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Invited Review

Haemonchus contortus: the then and now, and where to from here?

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ABSTRACT

Haemonchus contortus (Barber's pole worm or "BPW") is the nematode "nemesis" of small ruminant production systems in tropical and subtropical regions of the world. Its reputation derives from a combination of high fecundity and a short generational interval that provides an enviable developmental plasticity for adaptation or resistance to control measures. This review critically examines the historical and current literature on the host–parasite–environment interaction for *H. contortus*, particularly in sheep, to highlight changes in parasite distribution and ecology on pasture, changes to the seasonal inhibition of fourth stage larvae and the most appropriate models to identify protective responses and assess vaccines. The review also proposes pathways to bring host genetics to fruition and avenues where advances in the parasite genome may complement control measures.

1. Introduction

Haemonchus contortus is one of the major livestock parasites in tropical and temperate farming areas, likely accounting in Australia for the greater proportion of the annual AUD 436 million in production losses and costs of nematode control in the sheep Industry alone (Lane et al., 2015). An abomasal blood-sucking nematode, the larger L5 and adult parasites may remove up to 30 µl of blood per day, rapidly causing anaemia and subsequent death even before the pre-patent period, when infestations of >500 worms are present. With an establishment rate in single infections in naïve recipients of approximately 60% (Dineen et al., 1965), an impressive daily egg output of between 5000–15,000 eggs per female worm, the shortest patent period (circa (ca) 15 days) and life cycle (20 days) of any gastrointestinal nematode (GIN), the rapid build-up of infective *H. contortus* L3s (*Hc*L3s) on pasture under suitable climatic conditions has been assured. Consequently, the genetic variation contained on the average sheep paddock is guaranteed to be sufficiently high to permit rapid adaptive changes to climate, hosts and treatments (Anderson et al., 1978; Kaplan, 2004; Troell et al., 2006; Rose et al., 2014). Additionally, *H. contortus* can reproduce in several other livestock species including calves and goats, and undergo limited development in a variety of wildlife species including rabbits. Even with a preference for the warmer climates with erratic or seasonal rainfall and the associated risk of death of pasture L3s, such a fecund parasite can afford an "all or nothing" approach to survival of free-living stages in extremes of heat, cold or intra-host competition from other nematodes. However, in mixed abomasal infestations, *Hc*L3 establishment is compromised (Turner et al., 1962; Reinecke et al., 1981; Dobson and Barnes, 1995); an outcome countered by *H. contortus* with both enhanced fecundity and a shorter prepatent period (i.e. greater "virulence" – Poeschel and Todd, 1972a,b; Quigley, N.T., 2007. Virulence determinants of *Haemonchus contortus*. BSc (Vet) Thesis, University of Sydney, Australia; Hunt et al., 2008).

To address and counter increasing anthelmintic resistance in Australia, the original versions of integrated parasite management (IPM) programmes such as "Wormkill" were erected to utilise all aspects of the interactive elements of host, parasite and environment (HPE) to broaden control measures, reduce the frequency of chemical treatments and prolong the effective life of a limited range of anthelmintic treatments (Morley and Donald, 1980; Barger, 1997). These IPM programmes sought to mitigate the impact of parasitism by reducing the availability of L3s on pasture, so as to minimise worm intake and facilitate the development of immunity. Suppressive drenching regimes detracted from the development of immunity and left recipients susceptible in coming seasons (Barger, 1997). Additional elements of the IPM

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programmes included adequate nutrition to pregnant ewes and weaners (especially protein and trace elements; van Houtert et al., 1995; Donaldson et al., 1998; McClure et al., 1999; Strain and Stear, 2001; Macarthur et al., 2014) and selection or breeding of genetically resistant stock (Bishop, 2012). Both of these approaches enhanced the development of worm immunity which underpinned nematode resistance (Emery, 1996), while superior nutrition increased resilience (the ability to produce while parasitised). These were remarkably successful in the short term with up to 60% of producers adopting "Drenchplan" and "Wormkill" in regions with *Hc*L3 presence. However, as far as chemical resistance was concerned, by minimizing the numbers of pasture L3s (termed "spatial refugia"), IPM programmes unwittingly augmented selection for chemical resistance (Kaplan, 2004; Dobson et al., 2011). This was most profound with *H. contortus*, where high fecundity resulted in high levels of intergenerational mutations for selection. As animals were drenched, only the progeny of worms surviving the treatment contributed to the next generation of parasites on pasture. A timely reconsideration was necessary and newer iterations of "Wormkill" advocate a constant monitoring of pest/parasite, recent and predicted weather and stage of the production cycle so that the best decisions are implemented over time. In addition to manage resistance, several "targeted" drenching programmes to increase the numbers of refugia (parasites not exposed to the relevant chemical) have been modelled and trialled (Besier et al., 2010; Dobson et al., 2011; Cornelius et al., 2016). For *H. contortus*, the original refugia-based controls were introduced against benzimidazole-resistant *H. contortus* (Van Wyk and Van Schalkwyk, 1990) and became refined as the FAMACHA™ programme (Kenyon et al., 2009). However due to the parasite's ability to cause disease and death rapidly, the broad-acre farming practices, and the greater susceptibility to *H. contortus* of the Merino breed renders FAMACHA unsuitable for use in Australia. The most appropriate refugia-based strategy for this parasite is not defined, but requires solutions which are customised for region and enterprise.

This paper reviews the HPE interaction for *H. contortus*, documenting particularly those properties of the parasite which have permitted adaptations to climate, pathophysiology, immunity and control measures. Particular areas of importance include developmental and distributional changes to climate, alterations to the inhibition of L4 stages, and models most suited for mechanistic studies of immunity as well as vaccine development, formulation and assessment of efficacy. The review draws heavily on studies in sheep from Australia and deliberately presents historical studies that illuminate the HPE interaction to provide research directions. Advances in host and parasite genomics are also presented relative to knowledge of host immunity to identify areas for future research and expedite possibilities for new or updated control measures.

2. Changes in the parasite–environment interaction

2.1. Changes in the distribution of H. contortus in Australia

Averaged annual mean maximum and minimum temperatures are increasing across most of Australia with an associated statistically significant decrease in the annual occurrence of cold nights and cold days. Other temperature measurements display similar trends consistent with warming including: reductions in frost days and cold spells, and an associated significant increase in all the other temperature indices, particularly the annual occurrence of warm nights and warm days (Alexander et al., 2007). The average maximum temperature has risen by 0.7 °C and the minimum temperature by 1.1 °C in the non-seasonal rainfall zones of eastern Australia that are associated with outbreaks of haemonchosis with much of this warming occurring since 1950 (Alexander et al., 2007). Related to changes in climate as well as temperature tolerances, Fig. 1 illustrates in southeastern Australia, the increased spread of *H. contortus* over the past 70 years from the 1930s (Clunies Ross and Gordon, 1936) when compared with current data from larval cultures in 2015. However, as the climate changes, changes in the sheep industry have resulted in a reduced incidence in central Queensland. The predictive modelling is a first step towards a closer analysis of factors governing and predicting future movements of *H. contortus*.

2.2. Influences of changing temperatures affecting development and survival

Crofton and Whitlock (1965a,b) have shown that the smaller the egg, the less time it takes to hatch, while larger eggs can hatch at lower temperatures. However, below a minimum volume, which increases as temperature falls, eggs will not hatch at all. There must be selection for eggs close to this limit in size because less resources are required for smaller eggs and this is important for a parasite laying between 5000 and 12,000 eggs per day. This efficiency in egg production would be counter balanced by those *H. contortus* laying larger eggs that can hatch at lower temperatures. It appears that the parasite is following this strategy as Le Jambre et al. (1970) found that with a single isolate four different *H. contortus* vulvar phenotypes each had different mean egg sizes and Le Jambre (1972) reported that the eggs of each of these phenotypes had a different optimum temperature for development. There is also variation in the minimum temperature for development from egg to L3 between geographically different isotypes. A New York (USA) and a New England (Australia) isolate both could develop at 11 °C while an isolate from Louisiana (USA) required 13 °C (Le Jambre, 1981). Consequently, there is variation in temperature required for development, so that a response to selection could be indicated by either an increase in egg size or a decrease in minimum temperature for development. Taken together with warming temperatures (Alexander et al., 2007), these worm-specific and external factors could underscore the increased westward and southern distribution of *H. contortus* in Australia depicted in Fig. 1; the influences of rainfall and temperature on *H. contortus* distribution are generally consistent with the results of Southcott et al. (1976). Species distribution modelling (SDM) would provide some guide to future movements of *H. contortus* based on climatic predictions and host movements (Besier et al., 2016).

2.3. Seasonal epidemiology of H. contortus infestations

It is apparent that the survival strategy of *H. contortus* has changed during the past 30+ years from one of overwintering in the host as inhibited larvae (Barger et al., 1985) to one of completing development as soon as ingested. Previously, the main trigger for initiating inhibition of development was length of time larvae spent between completing development to infective stage until being ingested by the host. This age conditioning of larvae can be seen in the response to storage at 4 °C (Table 1) where the proportion of the 1971 isolate of *H. contortus* that became inhibited increased for the first 10 weeks of storage and then began to decline (Dash, 1971). Dutch field strains of *H. contortus* similarly have been induced into inhibited development by storage at 15 °C (Eysker, 1981; Hendrikx et al., 1988). Host resistance also plays a role in inducing inhibition of development in *H. contortus* larvae. Dineen et al. (1965) found increasing proportions of inhibited *Hc*L4s in sheep given daily infections of fresh larvae and slaughtered 28, 48 and 133 days after larval dosing first

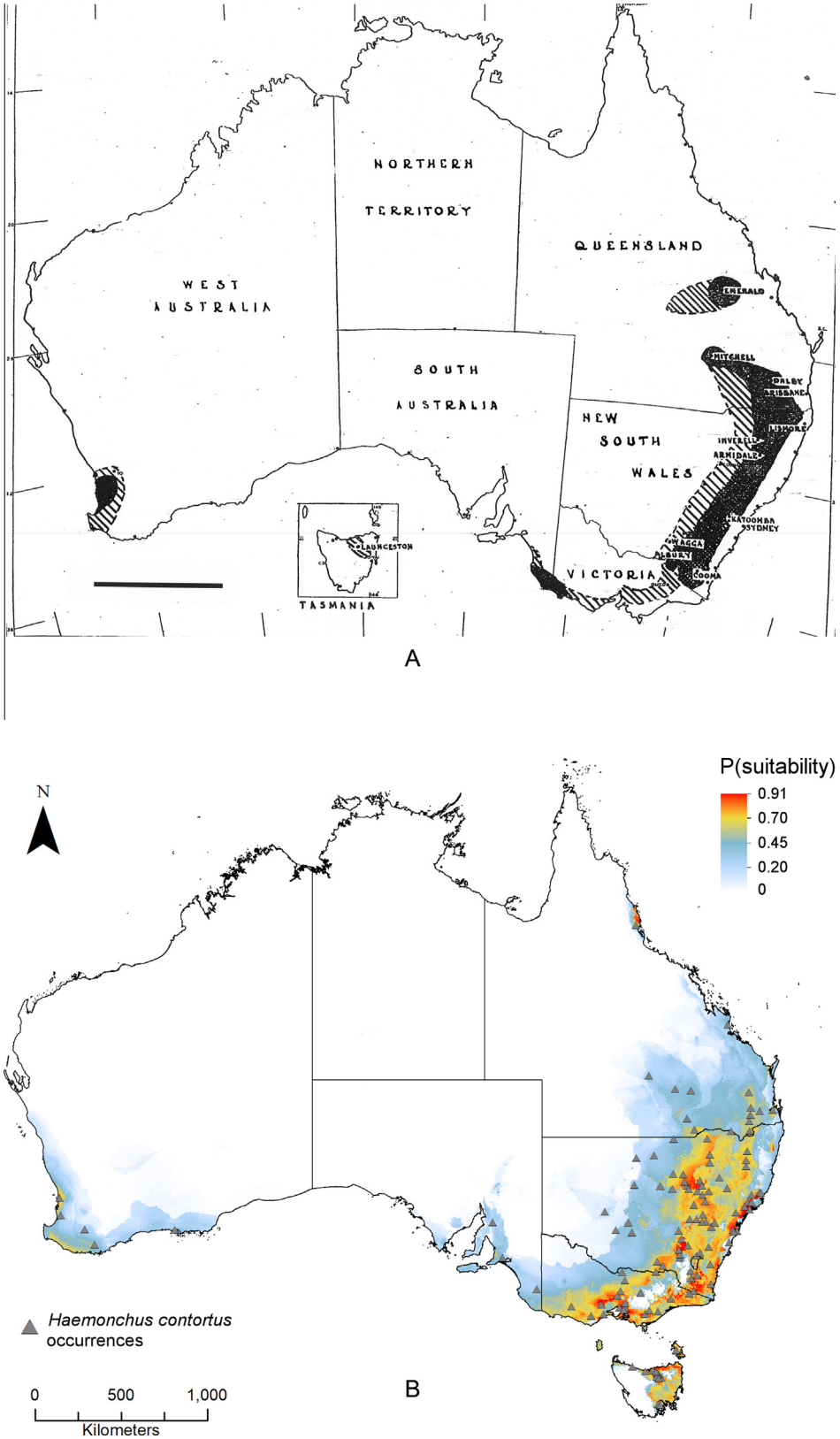

Fig. 1. The changing distribution of *Haemonchus contortus* in Australia. (A) Distribution of *Haemonchus contortus* in 1936 (adapted from Clunies Ross and Gordon, 1936; published with permission). Consistent and sporadic outbreaks of haemonchosis are indicated by the dark and shaded areas, respectively. Scale bar = 800 km. (B) Predictive distributional modelling of *H. contortus* based on climatic variables (produced by Dr. Nathan Emery). The dataset is available at https://data.mendeley.com/datasets/z36kfdtw9b/1. Triangles indicate the current distribution of *H. contortus* outbreaks sourced from larval cultures submitted to diagnostic laboratories throughout 2014–2015. These were used to validate the model. P (suitability) refers to the probability of the habitat being suitable based on 19 long-term average bioclimatic variables that were downloaded from www.worldclim.org (Hijmans et al., 2005). The MaxEnt AUC was 0.937 which means the model performed above average. From the modelling, the most important climate factors contributing to the predicted distribution of *H. contortus* were: precipitation of the driest quarter (49.9%); precipitation of the coldest quarter (15.6%); precipitation of the driest month (9%). The variables that contained the most unique data on their own were: annual precipitation, minimum temperature of the coldest month. There is a higher probability of *H. contortus* occurring in areas with 700–1200 mm annual rainfall.

Table 1
Effect of storage at 4 °C on the subsequent development of *Haemonchus contortus* larvae in sheep.

Weeks of storage	No. larvae given	No. worms recovered	% Inhibited larvae
5	5200	1750	3
9	5000	789	63
13	4900	2082	59
17	5000	575	52
21	5000	360	14

commenced. The increasing proportion of inhibited larvae over time was attributed to the gradual development of resistance during the continuous exposure to incoming larvae.

Consequently, there are two components contributing to the accumulation of inhibited larvae in sheep at pasture during autumn and winter. Firstly, *H. contortus* eggs, which require a minimum of 10–11 °C to hatch, stop developing as the temperature lowers during autumn. Larvae remaining on pasture are also ageing and becoming predisposed toward inhibition. Secondly, during late summer and early autumn, spring born lambs are beginning to develop immunity to infection and that also contributes to the likelihood that a larva will undergo inhibition. However, as discussed below, drenches capable of removing inhibited *Hc*L4 stages have altered the outcomes of this proclivity for inhibition.

In Australia, an increasing proportion of the spring larval intake become arrested submucosally as *Hc*L4s over summer coinciding with the peak in adult worms. The summer peak of both adults and larvae then decrease in early autumn (McKenna, 1973; Southcott et al., 1976; Smeal et al., 1980; Barger et al., 1985). Subsequent autumn and winter infections are overwhelmingly inhibited larvae. These larvae normally last until spring when either they resume development, or the intake of infective L3s from the pasture, no longer programmed to inhibit due to ageing (Eysker, 1981), produce either a "self-cure" (Stewart, 1955; Blitz and Gibbs, 1972b; Adams, 1993) or a spring rise in egg count (Barger and Le Jambre, 1979). This seasonal inhibition of development at the early L4 stage has been reported for *H. contortus* from many parts of the world (Blitz and Gibbs, 1972a,b; McKenna, 1973; Waller and Thomas, 1975; Southcott et al., 1976; Waller et al., 2004; Sargison et al., 2007). As described by these authors, the phenomenon appears to follow a similar pattern in all localities, from mid-summer onwards increasing numbers of ingested larvae are arrested in their development at the early L4, until by autumn virtually all of the recently established larval intake is being inhibited. These larvae resume their development some months later, so that inhibition of development appears to be an adaption to ensure the survival of the parasite over winter, when environmental conditions are unfavourable for its transmission (Blitz and Gibbs 1972a; Southcott et al., 1976). Occasionally the maturation of previously inhibited larvae has precipitated outbreaks of winter haemonchosis (Gibbs 1965; Wilson and Samson, 1970) but mainly it is implicated in the periparturient rise in the faecal worm egg count (FWEC) of spring-lambing ewes (Procter and Gibbs, 1968; Southcott et al., 1976; Waller et al., 2004; Sargison et al., 2007). The relative importance of inhibited development to the epidemiology of haemonchosis varies according to climatic conditions. Thus, Waller et al. (2004) concluded that under Swedish sheep farming conditions, *H. contortus* has evolved to survive the long, cold winters entirely within the host as the arrested larval stage, relying on the lambing ewe to complete its life cycle. Barger and Le Jambre (1979) demonstrated that even in the mild climate of the Northern Tablelands of New South Wales (NSW), Australia, where *H. contortus* larvae can overwinter on pasture, inhibited larvae were of equal importance in initiating a spring rise in FWEC.

2.4. Changes in the seasonal epidemiology of Australian H. contortus infestations

However, in recent Australian reports there has been no mention of inhibited *Hc*L4s being found during post mortem worm counts. There are two possible causes: (i) they are being overlooked or (ii) they are not present. In 1971–72, a field study was conducted on infested pasture at Armidale, NSW, Australia (Dash, 1971). During the next 12 months, the paddock was grazed continually by weaner sheep and another group of weaners that were worm-free until two were placed on the paddock for 2 weeks before being replaced by another two worm-free sheep. After grazing for 2 weeks, the two worm-free and two from the continually grazing flock were then kept in an animal house for a further 2 weeks before slaughter for worm counts. Observations on each plot continued for 12 months. The results indicated that *Hc*L4s entered into arrested development during autumn and winter (Fig. 2) when there was little chance of any eggs developing to infective larvae on pasture. The peak of inhibited worms occurred in previously worm-free animals as well as those who were continuously grazing. Consequently, inhibition over winter was not due to the host becoming resistant.

To address the lack of inhibited *H. contortus* larvae in present day post mortem reports, Le Jambre and Colditz (2006, unpublished data) repeated the initial study by Dash (1971). In this study, lambs were grazed with their dams until weaning when the ewes were removed from the paddock while the lambs continued grazing. Prior to lambing, two ewes and two lambs were euthanised for worm counts each month; after lambing two lambs were taken

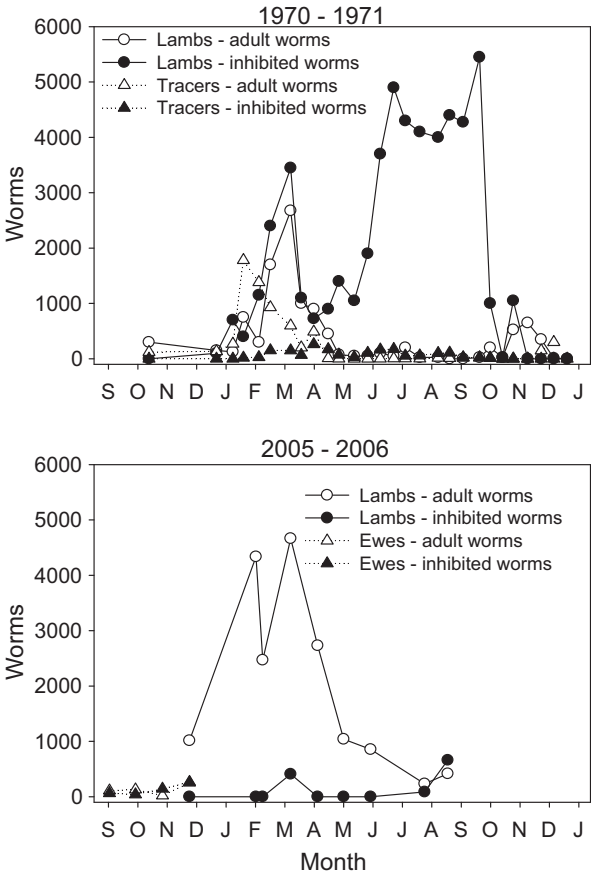

Fig. 2. Worm counts from necropsied sheep at intervals over a year (September 1970 to January 1972; September 2005 to January 2007) of grazing parasite-infested pasture. Once taken off pasture, sheep were housed for 14 days prior to necropsy. L4s found in these sheep were considered inhibited.

each month for worm counts. Post mortem worm counts from two sheep were done each month from a flock grazing at pasture over 1 year. Prior to euthanasia, these sheep were kept in an animal house with slatted floors for 2 weeks to allow any recently ingested larvae to develop to adults if they were not to become inhibited. The results of this study are compared with the data from 1970–71 in Fig. 2.

It can be seen from Fig. 2 that there are almost no inhibited *Hc*L4s in the 2007 study and it is completely different from the results of Dash's 1971 study. To determine whether this lack of inhibited larvae during winter was widespread, Le Jambre and Colditz (2006, unpublished data) tested four isolates of recently collected *H. contortus* and one isolate collected in 1981. All strains were frozen in liquid nitrogen within two passages of being collected. Upon thawing, sheep were dosed with the isolates and once the infection was patent, larvae were cultured from faeces and stored at 10 °C for 9 weeks to induce inhibition. Two worm-free lambs were each infected with one of the conditioned larval isolates. Thirty-five days following infection, the lambs were slaughtered and the abomasum scraped and digested with pepsin to collect adult and larval nematodes. The results of this post mortem worm count in Fig. 2 indicate that in both 1971 and 2006, adult worm burdens increased during summer to reach a peak in February. The big difference between 1971 and 2006 was in the inhibited portion of the populations. In 1971, there was a peak in inhibition in February coinciding with the adult worm peak and another peak in winter when the entire population was inhibited. In 2006, there was only a small peak coinciding with the peak of adult worms and no inhibited larvae in winter. That this change in epidemiology was widespread is supported by the results in Table 2. This has a profound impact on the seasonal occurrence of Australian haemonchosis; in 1971 it was a disease that was restricted to the warmer months, today it can occur in winter as well.

Consequently, the warming trend documented in Australia would seem to provide one of the necessary ingredients in a survival strategy that has shifted from over wintering in the host to one of attempting to complete the life cycle any time during the year including the cooler months. However, it is unlikely that the increased temperature has been the main factor for the epidemiological change in haemonchosis. Rather, it is more likely that the introduction of modern anthelmintics with their ability to kill inhibited larvae brought about the change. Inhibited larvae spend months in sheep without reproducing while being exposed to the likelihood of removal by anthelmintics. It appears that the conditions are right for a change in the distribution of *H. contortus* (see Fig. 1). Due to the presence of eggs in the faeces throughout the year, selection is now being applied to develop at lower temperatures and perhaps a reduced prepatent period. Is there variation in hatch and development on which selection can work? Given the developmental plasticity (Gilbert et al., 2015) and fecundity of *H. contortus*, this is entirely likely (see Section 3.2).

2.5. Parasite population genetics: strategies and applications

Similar to all eukaryotic organisms, *H. contortus* populations are subject to selective pressures in their environment which can favour or discriminate against certain variants in gene sequence (alleles). The study of population changes has typically two areas of interest; firstly in the variance of observed traits or phenotypes and secondly in the degree to which these phenotypes are genetically determined. For *H. contortus*, a major focus has been upon anthelmintic resistance, but many other phenotypes are important in the HPE interaction. Phenotypic variation in virulence (including infectivity and reproductive success) are obviously important (see Section 3.2) as is development or survival of *Hc*L3s in the field. That different isolates have differing phenotypes when analysed under identical environmental conditions, indicates that the observed phenotypic variations are genetically determined. A second method of detecting genetically determined variation is to place selection pressure upon the population to produce a divergent phenotype and assess its presence after the selection pressure has been removed. Le Jambre and Royal (1977) artificially selected *H. contortus* for phenotypic variation in the morphology of the vulval region and the "smooth" phenotype persisted. Similarly, to examine drug resistance, Sarai et al. (2015) used levamisole to generate significant resistance within the susceptible *H. contortus* population, "Kirby1981", from generation 6 onwards. Applications for these new phenotypes are obvious to define molecular markers to elucidate whether resident *H. contortus* populations on properties are developing resistance to particular anthelmintics. Lack of particular resistance markers could enable choices of efficacious chemicals. This is currently performed through a faecal egg count reduction test (FECRT) or larval development assay (LDA), however a DNA-based test would offer a standard platform for a range of drugs for a substantially reduced, cost, time and effort. Internationally, much research of genes and genomes has been applied to discover resistance markers for DNA tests (Kotze et al., 2014; Kotze and Prichard, 2014). For the oldest drug group still in use, the benzimidazoles (e.g. albendazole, oxfendazole, fenbendazole), a relationship between three alleles of the B-tubulin 1 (BTUB1) gene and resistance has long been established (Kwa et al., 1993), and DNA-based tests have been used to determine the resistance status of parasites within livestock farming regions (see Walsh et al., 2007; Corley and Jarmon, 2012; Shamaila et al., 2014). There are some salutary reminders that resistance determinants are not the same in all populations of a particular species. In an Australian isolate of *H. contortus*, two BTUB1 alleles, determined by single nucleotide polymorphisms (SNPs) affecting codon 200 (F200Y) and 198 (E198A) are involved in resistance, with the E198A allele conferring a higher level of resistance (Kotze et al., 2012). When two benzimidazole-resistant isolates from Australia and one from South Africa were analysed, the E198A and F200Y alleles were never found within the same DNA sequence (Ghisi et al., 2007). In contrast in Canada, 16 *H. contortus* populations from sheep did not possess the BTUB1 allele E198A, but allele F167Y was present in 12 of the populations, and F200Y was present in all 16 (Barrere et al., 2013). Each of the three BTUB1 alleles is predicted to alter the binding of benzimidazole drugs to the BTUB1 protein, but investigations of resistance against other anthelmintics has revealed a more diverse set of resistance mechanisms. For macrocyclic lactones (MLs; ivermectin, moxidectin, abamectin), changes to target sites, drug efflux pathways and other mechanisms have all been suggested for ML-resistant *H. contortus* (Williamson et al., 2011; Urdaneta-Marquez et al., 2014; Godoy et al., 2015), and for other parasites. As one example, a whole genome sequencing approach in wild yeast (*Saccharomyces cerevisiae*) found a large diversity of resistance mechanisms against multiple compounds (Ehrenreich et al., 2012). A major problem for discovery of causal genomic changes underlying phenotypic variation is that populations diverge from one another over time by genetic drift, founder effects, migration, mutation and selection. These forces are equally important for *H. contortus* (Gilleard and Redman, 2016). When two

Table 2
Inhibited *Haemonchus contortus* L4s found in recent isolates from Australia.

Isolate – Location and year of isolation	Mean adult worms	Mean L4s	% Inhibited
Bundarra2001 (VHR-23)	4586	0	0
Chiswick1981 (Wild #961)	3073	197	6
Wallangra2003	4190	0	0
Guyra2007	1717	0	0
Stanthorpe2007	4960	0	0

populations are studied due to an observed divergence in phenotype, this may not be the only difference between the populations, so that divergent alleles in the candidate gene is only a potential causative difference, not proof of the relationship between allele and phenotype. The only way to resolve this issue is to mix the two populations together, allow random mating, then re-select individuals which display the phenotype. Then, a continued statistical association between allele and phenotype provides firmer evidence of the causal relationship (as done by Ehrenreich et al., 2012). With *H. contortus*, genetic crossing experiments are possible by mixing divergent populations and selecting individuals displaying the phenotype of interest (Le Jambre et al., 1999; Hunt et al., 2010; Redman et al., 2012). These techniques are not yet developed for isolates of *Trichostrongylus* spp., justifying the use of *H. contortus* as a model for other parasitic nematodes of economic importance.

The study of causation between gene alleles and phenotypes is best achieved when the whole genome can be used as a "discovery" approach, rather than focussing on a particular subset of candidate genes. This removes selection bias, making the experiment more logical and statistically robust. Recent efforts to sequence the *H. contortus* genome have been undertaken to this end (Laing et al., 2013; Schwarz et al., 2013). Although the assembly of the *H. contortus* reference genome has been technically difficult, the existence of a collection of contiguous sequences of up to 947,000 bp in length (GenBank accession number HF955515) facilitates study of *H. contortus* populations and efforts are ongoing (Laing et al., 2016). Given the existence of two genome projects, genetic variation can be identified by directly comparing the two genome sequences (Fig. 3A). Currently, the genome scaffolds available publically are the consensus sequences and so do not illustrate any underlying genetic variation found within either of the genome projects. Secondly, genetic variants can be discovered by aligning expressed sequence tag sequences (ESTs) against reference genomes (Fig. 3B). These ESTs were produced some time ago (available from www.nematodes.org/nematodeESTs/nembase.html) and information is limited to the transcribed parts of the genome. Thirdly, new genomic sequence can be generated from isolates of interest and compared with the reference genome (Fig. 3C). This allows genetic variation to be evaluated, and permits assembly of a marker set distributed across the majority of scaffolds for delineating many genotype-phenotype relationships. This is a major advance allowing much more comprehensive investigations than those possible with older technologies such as microsatellites (Otsen et al., 2000; Roos et al., 2004; Redman et al., 2008, 2012; Hunt et al., 2010).

In addition to the discovery of genetic variation described above, a range of investigations of gene families existing within the *H. contortus* genome has been undertaken, mostly towards discovery of drug or vaccine targets and include cytochrome P450 genes (Laing et al., 2015), kinases (Stroehlein et al., 2015) and aminopeptidases (Mohandas et al., 2016). This work is further reviewed in Gasser et al. (2016). Allied technologies have been developed for proteomic investigations (Hart et al., 2015), studies of gene expression through quantitative PCR (Lecova et al., 2015), heterologous expression of genes in drug discovery pipelines (Law et al., 2015) and other opportunities as discussed in Britton et al. (2016).

A very high level of genetic diversity is evident in *H. contortus* (Kaplan, 2004; Gilleard and Redman, 2016). This was perhaps first observed when drug-resistant individuals were selected for five generations from a starting population and compared using 224 amplified fragment length polymorphism (AFLP) markers (Otsen et al., 2001). A decrease in allelic diversity following selection may have been expected, but this was not evident, showing that the genetic diversity in the starting population was extreme. A subsequent inbreeding experiment, using the progeny of single females to establish each subsequent generation, did achieve a level of inbreeding after 15 generations. This indicated that populations with lower levels of diversity can be produced (Roos et al., 2004), showing that it is possible to suppress *H. contortus* populations to levels which have a genetic impact. Restricting *H. contortus* populations on-farm could have a similar, but slower effect.

2.5.1. Further and future considerations of parasite population genetics

The association between phenotype and genotype is one aim of population genetics studies, but the elucidation of population differences can be informative in other ways. The degree of population structure can be inferred from genetic marker data, and studies have observed a modest level of structure (FST < 0.1) in *H. contortus* populations within continents (Hunt et al., 2008; Silvestre et al., 2009; Cerutti et al., 2010; Yin et al., 2013; Hussain et al., 2014), although there is a more marked divergence globally (FST > 0.1) (Troell et al., 2006; Redman et al., 2008; Yin et al., 2013). Low levels of structure imply a high level of gene flow due to population mixing (migration) across regions, so these observations are a warning that worldwide, *H. contortus* populations are not being limited genetically despite control efforts. Changes in drug resistance, virulence and environmental persistence occur by mutation and selection in situ, but also by the introduction of new genotypes through migration or local mutations. Clearly not all the mechanisms by which *H. contortus* are dispersed are understood (Hoberg and Zarlenga, 2016), and the relative importance of the differing mechanisms is also unclear. We must understand these processes before we can devise better systems to interrupt gene flow, nationally and within enterprises. The studies described above used a few markers to monitor population diversity and change, but extrapolations from a few loci may be misleading, ignoring significant changes occurring elsewhere in the genome. The need for higher density marker collections has been confirmed in many other areas of biology, including livestock animal breeding, so the technology for assembling such tools for *H. contortus* exists. Secondly, as illustrated in Fig. 3, the information necessary for predicting SNPs and other markers also exists. Finally, there is a need to attempt to track the exchange of individuals between *H. contortus* populations and changes within populations over time. This research needs a high density collection of genetic markers.

3. Changes in the host–parasite relationship

3.1. Nematode competition in the abomasum

Field infections with GIN are usually mixed, with additive pathogenic effects when parasites occupy different niches in the intestinal tract (Turner et al., 1962). However the population dynamics of abomasal parasitism causes suppression of *H. contortus* establishment. Turner et al. (1962) reported >90% reductions in *Haemonchus* burdens in sheep necropsied 3 months after dosing orally with 10,000 *Hc*L3s, 25,000 *Teladorsagia circumcincta (Tel)* L3s and 25,000 *Trichostrongylus axei* L3s. The numbers of *T. circumcincta* and *T. axei* were unaffected (Turner et al., 1962). The establishment and fecundity of *H. contortus* was also reduced more than 50% when trickle infections of 6000 *Tel* L3s and 3000 *Hc*L3s per week were given to lambs for 10–13 weeks (Dobson and Barnes, 1995). The effect required the presence of both parasites for the population control through changes in the abomasal physiology (increased pH), but some cross-protective immunity also reduced *Hc*L3 establishment in lambs previously infected with *Trichostrongylus colubriformis* (*Tc*) L3s (Barger, 1988; Adams et al.,

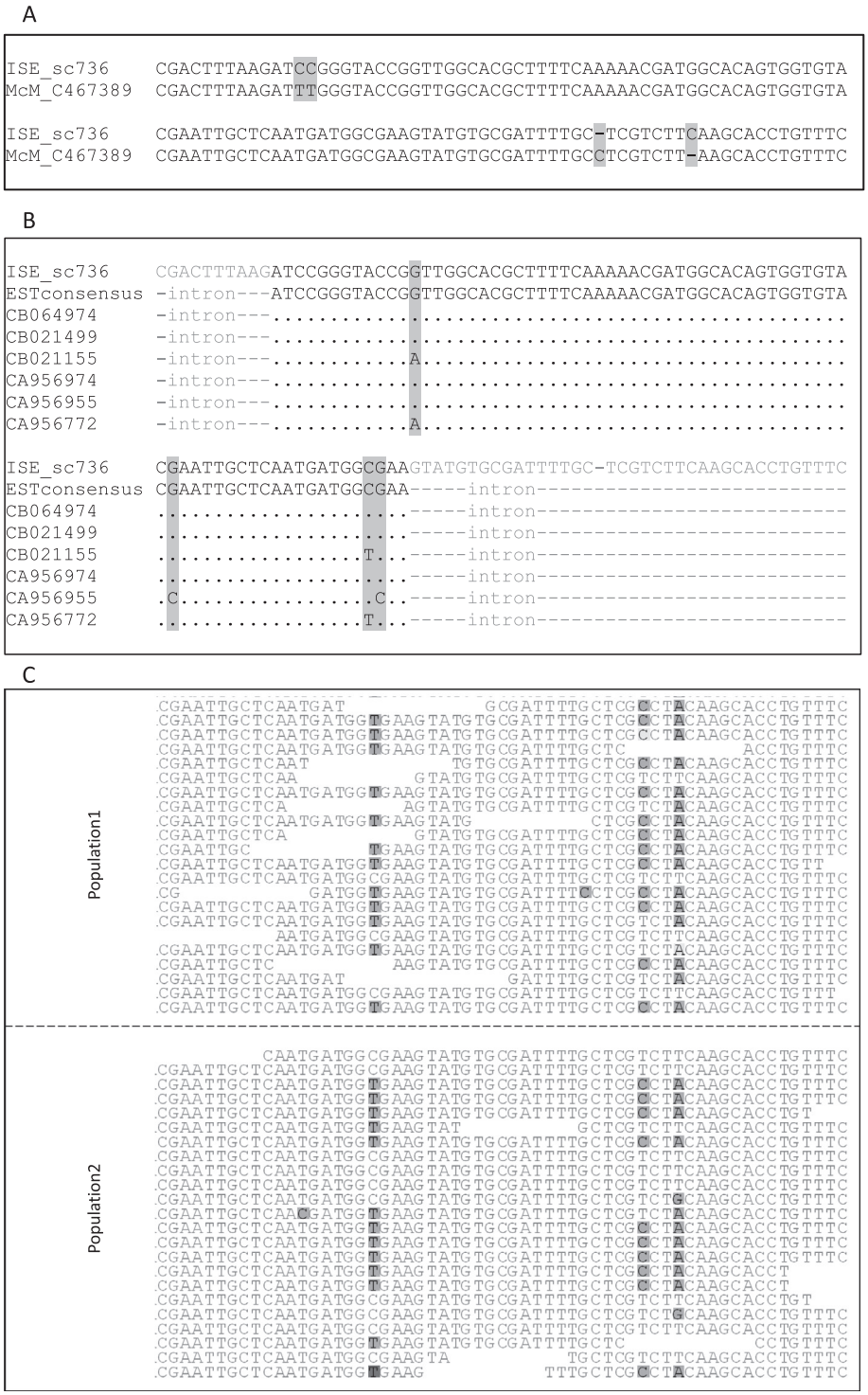

Fig. 3. Sources of information for discovering allelic variation in *Haemonchus contortus*. Three examples provided from alignments of portions of the GTP binding protein gene (GenBank accession number CDJ96183). (A) Alignment between scaffolds from two genome projects. ISE_sc736 is scaffold 736 from PRJEB506 (Laing et al., 2013). McM_C467389 is scaffold C467389 from PRJNA205202 (Schwarz et al., 2013). The shaded base pairs illustrate two substitution single nucleotide polymorphisms (SNPs) and two 1 bp insertion/deletion events. (B) Alignment of six expressed sequence tag (EST) sequences, delineated by GenBank accession code, against the ISE_sc736 genome sequence, and including a consensus EST sequence (Parkinson et al., 2004). Two transition (G/A, T/C) and two transversion (G/C) substitutions are shown in grey. (C) Alignment of short read Illumina sequences from two unpublished projects against ISE_sc736 (unpublished data). Three SNPs are indicated by darker grey shading in both projects, two transition substitutions (T/C) at positions 1566 and 1543 and one transversion (T/A) at 1540. A fourth possible SNP (T/C) at 1572 is indicated in population 2. For brevity, the alignments shown do not cover the full depth of sequence aligned. This type of data is useful as predictions of allele frequency can be made, and there is a better chance of distinguishing between sequence errors and allelic variation. For example, the alternate base pair, C, from the SNP at position 1572 is most likely a sequencing error, as it is present only once in 56 reads which cover this nucleotide in the alignment. In contrast the reference allele counts over the total read depth for the other three SNPs are: SNP 1566 22/59, SNP 1543 20/54 and SNP 1540 14/56 for population 1; and SNP 1566 23/53, SNP 1543 26/62 and SNP 1540 18/65 for population 2.

1989). This provides a basis for the distinct possibility to control *H. contortus* infestations by other nematode parasites. To this end, Reinecke et al. (1979), reduced by >50%, the establishment of *Hc*L3s from pasture, when Merino lambs were grazed around 1 month after being dosed with 40,000 *T. axei* L3s. However, this level of protection is insufficient to control *H. contortus* and the approach using live priming or "vaccines" is impractical for commercial use.

On the other hand, ingestion of *Hc*L3s initiates the "self-cure" reaction which is detrimental to the survival of other abomasal parasites and GIN established downstream in the gut (Stewart, 1955; Emery et al., 1993). Therefore, it could be investigated whether successful immunisation against *T. circumcincta* or *T. axei*, using a subunit vaccine (see below) could exert the same effect on *H. contortus* in the abomasum and *T. colubriformis* in the jejunum.

Drenches that do not remove inhibited stages of *T. axei* or *T. circumcincta* could allow the arrested stages to influence *Hc*L3 establishment. Alternatively, if the detrimental effect on *H. contortus* survival is due to increases in abomasal pH (Dobson and Barnes, 1995), could drugs such as ant-acids or other pharmacological agents have any effect on this parasite without compromise to abomasal function? Alternatively, do farms with more alkaline water sources or soils suffer less from haemonchosis?

3.2. Competition, immunity and H. contortus virulence

The concept of "virulence" should refer to the relative capacity of a pathogen to cause disease and differs from "pathogenicity", which is the ability to cause pathology (Casadevall and Pirofski, 2001). Virulence in helminthic parasites such as *H. contortus* may be considered as a product of both infectivity (between-host) and appropriation of host resources (within-host) for reproductive success (Medica and Sukhdeo, 2001). This becomes relevant with mixed nematode infections, developing host immunity and chemical treatment, all of which place the host–parasite dynamics and selective processes in constant flux. Because *H. contortus* is pathogenic throughout its reproductive phase, its transmission potential is related to virulence (Medica and Sukhdeo, 2001) and is a function of fecundity (reproductive fitness) and larval survivability. Changes in host–parasite dynamics (such as immunity) that increase the marginal reproductive fitness of *H. contortus*, will favour selection for increased virulence (Porco et al., 2005). Anthelmintic treatment will also skew selection towards a higher rate of reproduction and hence a higher virulence, to secure the host resources necessary to ensure a higher fecundity and a reduced pre-patent period. *Haemonchus contortus* virulence is expressed as increased L3 survival, higher *Hc*L3 establishment and development in vivo, shorter pre-patent period and greater fecundity (measured as FWEC expressed on a per female worm basis). Several studies have examined and compared these parameters using geographical isolates of *H. contortus*. From 18 isolates in the USA, Poeschel and Todd (1972a,b) found associations between increased "pathogenicity" with higher egg production per female worm (from 24 h egg collections) and a trend for a shorter pre-patent period, but not a higher worm recovery at necropsy. In Australia, the "Kirby1981" and "McMaster1931" isolates of *H. contortus* were more fecund and therefore virulent by the above definition than "Wallangra2003" or "GoldCoast2004", but the most pathogenic, defined either as the greatest depression in haematocrit per worm or the greatest depression of wool growth per worm were "Wallangra2003" and "Kirby1981" (Hunt et al., 2008). Kirby1981 and McMaster1931 were more virulent than "H9", "Hcon5", "IV-20" or "Mox-FD" isolates (Quigley, N.T., 2007. Virulence determinants of *Haemonchus contortus*. BSc (Vet) Hons Thesis, University of Sydney, Australia) based on FWEC and sequential worm counts. Definitive markers for enhanced virulence have not been discovered but should eventuate from genomic developments (see Section 2.4).

3.3. Macro-evolution of the host–parasite relationship related to host immunity

Host resistance evolved as a dynamic interaction between developing host immune responses and increasing intakes of infective L3s. Therefore, the most appropriate models to examine the development of immunity and the host–parasite interaction with GINs are derived from either field (natural) infections, or those studies using trickle (continuous) infections with graded doses of larvae, particularly in comparisons of genetically resistant or susceptible animals or breeds. Doses of approximately 500 *Hc*L3s three times per week are suggested for Merino sheep, double quantities for other breeds, mimicking field infestations (Gaba et al., 2006). Large, single dose infections (>8000 *Hc*L3s) will ensure the generation of acquired immunity by exceeding the estimated 3000 L3s as the minimum quantum needed to ignite the immune response (Dobson et al., 1990). However, such doses surmount innate responses during primary infections, preventing analysis of subtle interactions and the escalation of immunity over 4–6 weeks that progressively prevents L3 establishment, arrests or inhibits L4 development, reduces fecundity and expels adult worms. Large challenge doses also compromise the ability of vaccine-induced immunity to deal with the lower levels of incoming L3s experienced on pasture (Gaba et al., 2006). Even in immune animals, some parasites may establish before the anamnestic immune response is activated (Smith et al., 1983; Dobson et al., 1990), while high intakes of infective L3s provide serious challenges to even the strongest immunity. Many studies of the "mac ro-interaction" have monitored infections using FWEC, but the most informative reports have utilised radio-labelled or drench-resistant L3s with sequential necropsy to provide worm numbers and larval establishment rates as indicators of developing immunity. Consistent with results for calves dosed with *Ostertagia ostertagi* (Michel, 1970), Barger et al. (1985) reported that sheep given three doses of between 200 and 1600 *Hc*L3s per week developed immunity which reduced L3 establishment to around zero within 6–9 weeks, with a concomitant increase in inhibited L4 stages. Adult worms were subjected to continual turnover depending on the L3 intake (Barger et al., 1985). The interval of 4–9 weeks taken to maximise the escalating level of immunity was similar to that observed when lambs were trickle infected with graded doses of *Tc*L3s (Dobson et al., 1990). Therefore, the seasonal epidemiology of the host–*H. contortus* interaction was regulated by larval intake causing increasing arrest of mucosal L4 stages (Barger et al., 1985; Barger and Le Jambre, 1988), but that a "self-cure" reaction, a type-1 hypersensitivity response incited by ingestion of *Hc*L3s in sensitised sheep, was responsible in part, for the elimination and turnover of adult parasites (Stewart, 1955), possibly also involving "immune exclusion" reported when using high dose challenges of immune sheep (Miller, 1984). The reaction also eliminated other abomasal parasites (*T. circumcincta* and *T. axei*) as well as operating distally to remove *T. colubriformis* from the proximal small intestine (Stewart, 1955).

The heritability (h^2) of reduced FWEC for *H. contortus* is around 0.3 (Windon, 1996) and egg counts are highly correlated ($r^2 > 0.7$) with numbers of adult female worms in the abomasa (Rowe et al., 2008). An understanding of the sequential analysis of worm establishment and the genetic basis of immunity in flocks or breeds of sheep has helped to define protective responses and suggest genetic and biological markers to enable genetic selection. Resistant sheep develop effective immune responses to reduce L3 establishment earlier, so reducing overall worm burdens and parasitic costs to production (Windon, 1996). These animals may possess

immune systems that react quantitatively or qualitatively to lower amounts of worm antigen have more effective innate immune responses to reduce early infections or activate acquired immunity to reject worms. Alternatively or additionally, they may remove worms with less collateral damage in the gut or have better foraging activity to increase dietary protein, which is essential for optimal development of worm immunity in ovine weaners (Coop et al., 1995; van Houtert et al., 1995) and abrogates the periparturient relaxation of immunity (PPRI) (Donaldson et al., 1998). The large component of "environmental" variation, together with the complexity of the host immune response to differing stages of the parasite and the measurement of FWEC as the phenotype some 3–4 weeks after initial infection, may account for the difficulties with finding reliable genetic and biological markers to allow for genetics strategies to be progressed. Although the heritability for resilience is around 0.1 (Albers et al., 1987), selection on-farm for indices of productivity (measured simply by survival, live weight gain or fleece parameters) could provide greater long-term protection from the ravages of haemonchosis.

3.4. Micro-evolution of the host–parasite interaction related to host immunity

To facilitate dynamic study of the local host response to *H. contortus*, the "microevolution" of the host–parasite interaction can be assessed at the site of infection through laparoscopic biopsy or sequential necropsy (Rowe et al., 2009). Recently, an elegant series of studies have examined daily, the development of host immune responses in cannulated lymph ducts, continuously draining abomasal or intestinal sites in susceptible and resistant sheep (see Hein et al., 2010). In naïve hosts, the establishment rates for a range of gastrointestinal nematodes range from 40–80% (Dobson et al., 1990) and are <50% for *Hc*L3s (Barger et al., 1985). The initial losses are caused by innate inflammatory responses, as rates are enhanced by pretreatment with corticosteroids (used to maintain donor sheep) (Miller, 1984; Emery, 1996) and may involve eosinophil activity (Hein et al., 2010). Eosinophil activity against *Hc*L3s is not surprising, as inoculation of soluble extracts of *Hc*L3s into the mammary gland of immune and non-immune ewes elicits within 24 h, an exudate comprising >90% eosinophils, confirming the chemoattractive activity of *Hc*L3 products (Adams and Colditz, 1991). At the local site, the subsequent expression of nematode immunity in ruminants is consistent with a Th2 "allergic" response with production of sensitised CD4+ lymphocytes expressing and secreting interleukins IL5, IL13 and TNFα (Pernthaner et al., 2005a,b; Ingham et al., 2008) and reduced expression of CXCL10 (Andronicos et al., 2010). The Th2 phenotype is evident amongst sensitised cells from intestinal and abomasal lymph from challenged and resistant sheep (see Hein et al., 2010). More effective resistance to helminth infections in ruminants is also associated with increasing levels of serum and mucosal, parasite-specific IgG1, IgA and IgE (Smith et al., 1983; Bendixsen et al., 2004; Pernthaner et al., 2005b; Hein et al., 2010). The effector component of immunity is consistent with an "allergic" inflammatory response involving mucosal mast cells and eosinophils (Stewart, 1955; Emery et al., 1993; Emery, 1996; Hein et al., 2010) that secrete inflammatory mediators which paralyse worm motility (Jones et al., 1994), prevent L3 establishment, suppress egg production, remove adult worms (McClure et al., 1992) and perhaps disorientate incoming L3s. Mucosal mast cells (MMC) isolated from the small intestine of immune sheep released protease, leucotrienes and histamine after incubation with *Tc*L3 extracts (Jones et al., 1992; Bendixsen et al., 1995) and substance P and leucotriene C4 inhibited migration of *Tc*L3 and *Hc*L3 in vitro at physiological concentrations found in the lumen of the ovine small intestine (Jones and Emery, unpublished data). Despite these strong associations with immunity and worm rejection, unequivocal evidence of a protective immune response has only been demonstrated for antibody to the carbohydrate larval antigen (CarLA) found on many nematode L3s (including *Hc*L3s). Antibody to CarLA inhibits L3 establishment in a neutralisation assay in vivo (Harrison et al., 2008). Unfortunately, *Hc*L3s express a single invariant epitope differing in structure from *Tel*L3s and *Tc*L3s and passive protection was not provided against *Hc*L3 establishment (Harrison et al., 2008). This has implications for prophylaxis and diagnostic aids to select *H. contortus*-resistant livestock.

Overall, the literature attests to the complexity of nematode immunity in livestock and the difficulties of defining protective responses to enable definition of genetic and bio-markers (other than FWEC). Definition of protective effector responses is an area which also needs substantial research input if vaccinal control using conventional worm antigens and operating by the "natural" acquired immune mechanisms is to be successful. Most attention has analysed gene expression and responses in the abomasal wall and lymph, while those effector mechanisms preventing establishment or removing incumbent abomasal worms are largely unappreciated, even though their detrimental effects on worm growth, maturity and fecundity are well documented (Stear et al., 1995; Nisbet et al., 2016). Identifying and analysing the functional characteristics of innate responses against *H. contortus* involving moieties such as galectin is one approach (Nisbet et al., 2016). While Barbervax (below) is highly effective against the blood-sucking parasitic stages of *H. contortus*, larval vaccines that might target early parasitism (Harrison et al., 2008) by one or several nematodes have been tantalizingly unsuccessful in consistently reducing larval establishment by >50% using single–dose or continuous challenges (Nisbet et al., 2016). Taken together with the rejection of approximately half of the incoming *Hc*L3s by steroid-sensitive (innate) mechanisms, kinetic analyses of this early rejection process and events during the induction of acquired immunity to *Hc*L3s in the abomasum and small intestine *in vivo* or *ex vivo* is urgently needed for more precise diagnostic and selection methods and markers, and to support vaccine formulation.

3.5. Host immunity: genetic strategies and applications

The analysis of sheep with differing levels of resistance to parasite infection has been a subject of research for more than 40 years, but current markers can only account for <5% of the heritable variation in FWEC (ca 30%). Some issues include a failure to acknowledge the biological limitations of using FWEC as the read-out some 3–4 weeks after the initial host–parasite encounter (above). Although technically more difficult, a better endpoint would be *Hc*L3 establishment, to align with mechanisms from resistant sheep (Windon, 1996). Other work has focussed on the use of new technologies without firm links to a broader discovery-to-delivery pipeline and nearly all the work has failed to appreciate the huge global diversity in livestock genetics and livestock production systems. Therefore, a new framework needs to make the findings of individual studies applicable to the international community. In the framework proposed in Fig. 4, an important first level of investigation is simply to observe the potential for improvement (Fig. 4A). In sheep, field comparisons with continuous infections have been used to identify superior resistance in Barbados Blackbelly, U.S. St. Croix, Florida Native and Gulf Coast Native breeds in the Americas, Indonesian Thin tail, Indian Garole in Asia, and African Red Maasai breeds (Windon, 1996; Mugambi et al., 1997; Amarante et al., 2004; Bishop, 2012). The detection of superior genetics is most useful beyond the breed being studied when the resistance character can be analysed separately from the other characteristics of the strain or breed (Fig. 4B). Out-crossing experiments and the use of selection lines can separate the parasite resistance/susceptibility

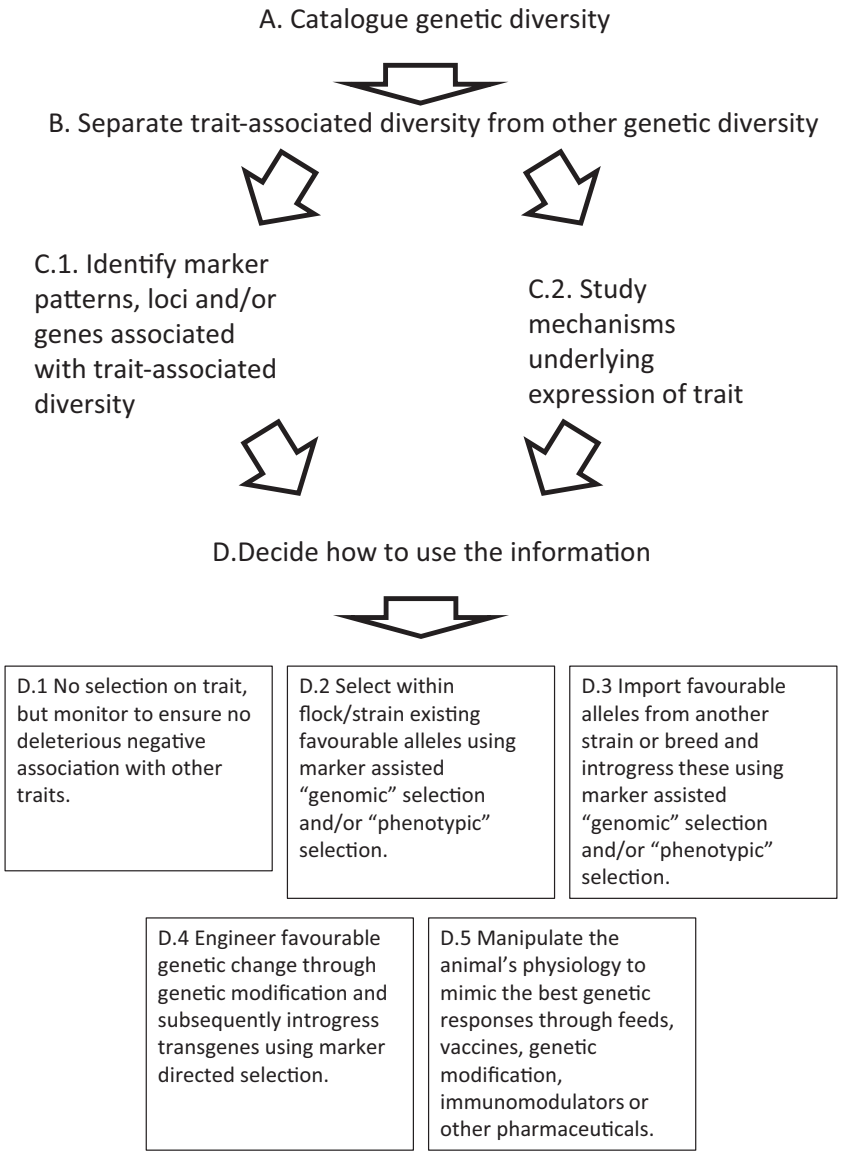

Fig. 4. Suggested pathway for discovery and application of genomic and genetic information, from discovering the genetic diversity (A), through evaluating the genetic component of variation (B), exploring the genetic and phenotypic characteristics of divergent individuals, lines or breeds (C) and finally deciding how best to use the research outcomes (D).

traits from other characteristics which may be fixed in the breeds due to founder effects, genetic drift and selection of chromosomally co-located alleles. For example, the Red Maasai and Dorper breeds were crossed and backcross progeny selected for study (Mugambi et al., 1997; Benavides et al., 2015). These types of breeding experiments are conducive to the non-biased subsequent discovery of genetic markers/genomic regions influencing resistance/susceptibility (Fig. 4C, path 1), and for the comparative study of the physiological and cellular mechanisms of resistance/susceptibility (Fig. 4C, path 2). Divergent selection lines of sheep have been used to discover genomic regions controlling resistance (Beh et al., 2002; McRae et al., 2014), to detect gene expression differences between lines (Diez-Tacson et al., 2005; Ingham et al., 2008; Andronicos et al., 2010), to analyse blood-detectable phenotypes or biomarkers for selection (Ingham et al., 2011; Andronicos et al., 2014) and to examine the interaction between nutrition and genetics (Doyle et al., 2014). It is imperative that applied research be undertaken to realise the benefits of the above research steps (Fig. 4D). A failure to deliver outcomes alienates livestock owners, research funding bodies and non-government organisations interested in livestock welfare. This is not a trivial exercise, especially for validation and registration of new actives and selection tools. In Fig. 4D, five differing paths towards utilising genomic and mechanistic information about parasite resistance in livestock are proposed. The choice of options will differ depending on the livestock species and strain/breed, the production system and the expectations of both regulatory authorities and consumers. It is sensible to realise these choices and their implications to guide applied research and development into the future.

Of the five proposed paths, the selection for resistant genotypes within the flock or breed (Fig. 4, path 2) has received most attention. The development of "genomic selection" systems for increasing resistance of sheep to *H. contortus* and *T. colubriformis* has been a focus. Kemper et al. (2011) attempted to both estimate the effects of a genome wide set of polymorphic markers on the response to parasites and to establish whether any of the identified correlations were applicable to parasite resistance breeding using genomic selection. Comparing the results of the linkage disequilibrium study from the 20 large single sire families with data from 316 industry sires across three strains of Merino, Poll Dorset, White

Suffolk and Border Leicester breeds, the authors identified three significant markers on *Ovis aries* chromosome 1 and one each on chromosomes 17 and 18. However, these markers only explained 4.73 ± 1.82% of the variance in *H. contortus* FWEC with an apparent false discovery rate of 74.8%. They questioned the value of selection using the identified markers but proposed that the use of all markers (48,640 SNPs) would be a better approach to try and capture more of the estimated 10–24% heritable variation in FWEC. The use of the total marker set to estimate breeding values had a 0.22 to 0.26 correlation (best estimates for terminal sire and merino sheep breeds) with estimates based on phenotypic assessment of progeny (progeny testing). However to date, this has not been attempted at an industry-wide scale. This low level of correlation would not be acceptable for medical or veterinary diagnostics, but the cost saving in genotyping sires compared with progeny testing makes the approach attractive despite the poor performance. In reality, progeny testing remains the mainstay of calculating estimated breeding values for selection, and may be assisted to some level by "genomic selection" in the future. The complexity of the host immune response to differing stages of the parasite and the measurement of FWEC as the phenotype some 3–4 weeks after initial infection, may account for the difficulty with finding reliable genetic markers that explain larger amounts of the genetic variation.

Selection on some production traits in livestock species has been very successful and the use of "genomic selection" to complement progeny testing has been implemented, for example in Holstein Friesian dairy cattle. Where this has occurred, problems remain with estimating parasite resistance breeding values. It may be possible to use the data from high-throughput genetic marker analysis to reduce the chance of negative drift in parasite resistance rather than as a tool for actively selecting resistant individuals. In Fig. 4 we propose this as path D.1. For example, maintenance of the favourable alleles of the five markers identified for *H. contortus* resistance by Kemper et al. (2011) could be achieved, despite selection using an index of traits not including parasite resistance. Alternatively, phenotypic data could be used to achieve the same end point, balancing the selection of sires for other traits such that the mean estimated breeding value (EBV) for parasite resistance was zero. Although this approach will not improve parasite control, it will at least attempt to maintain the flock at a constant level and this should in turn make the effectiveness of non-genetic control mechanisms, such as nutritional strategies, more constant over time.

The small gains achieved through genetic selection for parasite resistance within flocks or strains require a concerted effort over many years for effect (Woolaston and Piper, 1996; Morris et al., 1998; Woolaston and Windon, 2001). In reality, the proportion of the selection index dedicated to parasite resistance must be less than for production traits which have an economic weighting and sometimes a higher heritability. In these circumstances, a short-term introgression programme bringing in favourable alleles from a source outside of the strain or breed could be used and modern genetics offers a favourable proposition to achieve this rapid introgression with low risk (path D.3 in Fig. 4). For example, the Red Maasai is inferior to many other breeds for growth rate and wool characteristics, so introgression of the five loci with desirable parasite resistance alleles from the Red Maasai breed (Benavides et al., 2015) into a breed such as medium wool Australian Merinos, would be risky. However the identification of five small regions in the sheep genome on chromosomes 2, 6, 11, 12 and 15, means that these regions can be specifically selected in a crossbreeding scheme, whilst Merino alleles can be selected for the remainder of the genome.

This type of genomics-directed introgression is an attractive possibility, but comes with two provisos. First, the mechanism of the parasite resistance in the originating breed or strain must be known and exhibit a positive relationship with production traits (Albers et al., 1987). Second, a custom made genomic selection tool will be needed. Existing SNP-based tools, for example the sheep 50 k SNP panel, are biased towards markers where the minor allele exists at reasonable frequency (~10%) in all populations of interest. A marker used in an introgression project however, would work best if the minor allele was virtually absent from one of the two populations and ideally was the major allelic variant in the other population. These are the markers needed to ensure that the population into which the new genetics are being introgressed remains unchanged at the end of the crossbreeding programme.

The three paths to outcome outlined above make use of breeding as the major delivery tool. Another alternative potentially available is to create transgenic livestock capable of withstanding parasite infection (Fig. 4, path D.4). Expression-based mechanistic studies can suggest transgenes for parasite resistance, as can other work such as described in Section 3.4 and medical research on colitis and other pro-inflammatory intestinal conditions. These may seek to utilise genes involved in Th2 responses (Andronicos et al., 2012), but are more controversial and higher risk with the complexity of parasite resistance mechanisms. It is more likely in the short term, that transgenic studies will be used to confirm the importance of particular genes designated for selection indices. However, the creation of a successful transgene for making transgenic livestock capable of withstanding parasite infection would only be the first step towards an outcome. The production of sufficient transgenic animals to disseminate the transgene throughout a breed or strain would be the second step and a programme of marker-assisted introgression as described above would also be necessary before an appreciable impact on the industry as a whole could be achieved.

A fifth option exists for capitalising upon research focussed on the genetics and physiology of livestock genetically resistant to parasites (Fig. 4, path 5). Although the processes of population genetics have operated on livestock populations over millennia to produce some resistant genotypes, human enterprise, through livestock agriculture has changed the relationship between parasite and host over just a few centuries. By understanding the nutritional demands of effective immunological responses to *H. contortus* and balances with productivity, supplements may be tailor-made to prepare animals for predicted increases in parasitic larvae on pasture, to withstand parasite infections and to recover after parasitic infections have cleared. By understanding the cellular, paracrine and endocrine responses to parasitism which are effective, we could utilise and deliver orally to the gut, key moieties to drive an earlier, effective response to parasitism. This could exploit some of the huge amount of non-genetic influences contributing to variations in FWECs.

3.6. Host immunity to parasites: vaccines and prophylaxis

Vaccines to protect against *H. contortus* infection have had a long and complicated history. While effective at doses of approximately 20,000 irradiated L3s, the first generation of irradiated larval vaccines suffered from the cost of production (Smith and Angus, 1980) and the cold chain supply of live vaccines from laboratory to field. This is particularly problematic for smallholder livestock enterprises in developing nations. The same practical problems also apply to vaccination of young lambs with repeated small doses of 400 *Hc*L3s when a proclivity for Th2 responses persisted from gestation into the neonatal period. This protocol induced a moderate (ca 50%) level of protection against a trickle challenge (Emery et al., 2000). Vaccines utilizing a wide range of other (mainly larval) antigens from *Tc*L3 or *Hc*L3 have not yielded reproducibly, sufficient levels of protection to be commercially

viable (Emery, 1996; Jacobs et al., 1999; Meeusen et al., 2005; Nisbet et al., 2016). Arguably, efficacy needs to be higher (>80%) for *H. contortus*. As discussed, the lack of success is not associated with a lack of potential antigens but from an inability to design and deliver mucosal vaccines that induce protective immunity by the mechanisms involved in the normal host–parasite relationship (McNeilly et al., 2008). Two prerequisites are necessary: definition of the protective response to be targeted and continuous low dose (trickle) challenge over 4–5 weeks to allow the vaccine model to adequately represent field situations (Gaba et al., 2006) and the time needed to evolve immunity against incoming *Hc*L3s (Barger et al., 1985). The expression of inflammatory episodes in the gut is necessarily, highly regulated to local problem areas as evidenced by the characteristics of the effector response (Stewart, 1955; Emery et al., 1993), so that targeted mucosal delivery is an important consideration for induction of site-specific protection against helminths. For this approach, topical application of antigens in chitosan gels shows promise to induce local mucosal immune responses when applied over intestinal or rectal Peyer's patches (Senel and McClure, 2004; McNeilly et al., 2008). Adenoviral vectors expressing protein antigens have also proven effective to induce specific immune responses in neonatal lambs following enteric administration (Mutwiri et al., 2000).

Recently, and following the success of vaccines against the cattle tick *Rhipicephalus australis* (previously *Boophilus microplus*) using a concealed gut antigen Bm86 (see Willadsen, 2004), a similar approach has seen the momentous launch of Barbervax ™ utilizing the H-galGP gut antigen from adult *H. contortus* and prepared cost-effectively, from worms harvested ex vivo (Le Jambre et al., 2008). Several decades of research failed to produce a recombinant version of the original H11 complex which was protective (Munn et al., 1987; detailed in Nisbet et al., 2016), whereas the native antigen vaccine in Montanide adjuvant generated >80% reduction in FWECs (http://barbervax.com.au/). Similar preparations from gut membrane glycoproteins from *O. ostertagi* induced high levels of protection against challenge with *Hc*L3s in sheep, but not against *O. ostertagi* in calves (Smith et al., 2000). The H-galGP antigen complex appears conserved between geographical isolates of *H. contortus,* because the vaccine is effective across many areas of the world including South Africa, and multiple regions within Australia. The vaccine may be given to ewes to provide passive protection to at-risk neonates, but five doses at 4–5 week intervals, commencing at marking, is recommended to maintain levels of the specific antibody in the absence of boosting during normal infections. Labour and vaccine costs simply replace the numbers of musters and drenches given over the same period to weaners and combined strategies of drenches and vaccine have been recommended. It remains to be seen whether *H. contortus* populations will change genetically to withstand the hidden antigen vaccine, perhaps by changes to the sequence, structure or expression of the target antigens. The hatchability of eggs from surviving female worms could also be compromised as observed for the tick vaccine (Willadsen, 2004). However, Barbervax™ provides a timely and much-needed addition to integrated parasite control of *H. contortus* in Australia as resistance builds to MLs and monepantel drenches (Van den Brom et al., 2015; https://wormmailinthecloud.wordpress.com/2015/09/21) and the parasite is changing its range (Fig. 1; Besier et al., 2016).

4. Future considerations

From the issues discussed in this paper and elsewhere, it is evident that *H. contortus* is infamous for its ability to cause acute parasitism and death, rapid development of anthelmintic resistance, and an expansion of its range (in Australia) through a combination of adaptive changes. These adaptations include temperature tolerances for egg hatch and larval survival, both of which need more research effort to appreciate their bases and impact on the epidemiology of this parasite. It is possible that the genetic components of egg size, fecundity, egg hatch and L3 survival may be defined through advances in the parasite genome and the availability of a suite of informative genome-wide markers. Alternatively, since the increased spread of haemonchosis is largely permitted by changes in climate, there would be a concomitant change to seasonal pasture growth, perhaps also allowing some changes to the timing of lambing to avoid periods of high levels of *Hc*L3 availability or survival. These could be customised on-farm or regionally depending on the livestock enterprise.

IPM programmes have caused reductions in refugia and abetted the development of chemical resistance by parasites. To counter this effect, the Barbervax™ vaccine has been a welcome introduction, but under ideal conditions provides levels of protection approaching that of effective drenches. So the phenotype of surviving populations needs to be assessed. Additionally in sheep, targeted treatment (TT) regimes based on clinical presentation (e.g. Famacha™) and body weight have been shown to be effective to manage drench resistance in *T. circumcincta* with admirable worm control (Cornelius et al., 2016). However, due to the high fecundity and pathogenicity of *H. contortus*, these regimes have not been extended to the control of haemonchosis in large production enterprises. More research is needed to examine the sustainability of pasture *Hc*L3 control using programmes based on vaccination or TT.

Unwittingly, more effective drenches have orchestrated changes to the host–parasite relationship with the removal of inhibited stages, manifesting as year-round, clinical haemonchosis in some regions. Whether there is any residual role for rapid rejection or "self-cure" (Stewart, 1955) under these conditions is uncertain; whether host immunity is facilitated or retarded under the changed host–parasite epidemiology is also unclear. However, reliable reproduction of the self-cure response experimentally, has proven unexpectedly difficult (Emery, D., unpublished data).

The control of *H. contortus* on-farm requires short-term vigilance and monitoring, and longer-term planning through control programmes that involve the use of animals with superior nematode resistance or resilience. For the former, and despite its lack of sensitivity and correlation with worm burdens, the low cost of a FWEC accounts for its persistence as the decision tool for worm control. Molecular tools should assume more widespread use as costs decrease and will ultimately replace larval culture to assist with defining the presence and levels of *H. contortus* on pasture and in livestock. For the implementation of worm-resistant stock, endemic *H. contortus* has ensured that worm resistance features more prominently in selection indices, but the promise of genetic, gene expression and biomarkers to facilitate selection are yet to be fully realised. It is astonishing that the culling of highly susceptible, "worm factories" is not more widely practiced. For the parasite, a further avenue for molecular testing would be "customised profiling" using a genetic test to monitor the robustness of *H. contortus* populations. This could be used as a benchmark by which multiple control strategies can be compared, aiming for a decline in heterogeneity measured as reduced allelic diversity and effective population size as a good indication that management is having an impact on the population. In contrast, a finding of increased diversity or increases in allele frequency for minor alleles would constitute less successful population control, and should be a decision trigger for re-evaluating the parasite management regime.

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References

Adams, D.B., 1993. Systemic responses to challenge infection with *Haemonchus contortus* in immune Merino sheep. Vet. Res. Comm. 17, 25–35.

Adams, D.B., Anderson, B.H., Windon, R.G., 1989. Cross-immunity between *Haemonchus contortus* and *Trichostrongylus colubriformis* in sheep. Int. J. Parasitol. 19, 717–722.

Adams, D.B., Colditz, I.A., 1991. Immunity to *Haemonchus contortus* and the cellular response to helminth antigens in the mammary gland of non-lactating sheep. Int. J. Parasitol. 21, 631–639.

Albers, G.A.A., Gray, D.G., Piper, L.R., Barker, J.S.F., Le Jambre, L.J., Barger, I.A., 1987. The genetics of resistance and resilience to *Haemonchus contortus* infection in young Merino sheep. Int. J. Parasitol. 17, 1355–1363.

Alexander, L.V., Hope, P., Collins, D., Trewin, B., Lynch, A., Nicholls, N., 2007. Trends in Australia's climate means and extremes: a global context. Aust. Met. Mag. 56, 1–18.

Amarante, A.F.T., Bricarello, P.A., Rocha, R.A., Gennari, S.M., 2004. Resistance of Santa Ines, Suffolk and Ile de France sheep to naturally acquired gastrointestinal nematode infections. Vet. Parasitol. 120, 91–106.

Anderson, N., Dash, K.M., Donald, A.D., Southcott, W.H., Waller, P.J., 1978. Epidemiology and control of nematode infections. In: Donald, A.D., Southcott, W.H., Dineen, J.K. (Eds.), The Epidemiology and Control of Gastrointestinal Parasites of Sheep in Australia. CSIRO, Australia, pp. 23–51.

Andronicos, N.M., Henshall, J.M., Le Jambre, L.F., Hunt, P.W., Ingham, A.B., 2014. A one shot blood phenotype can identify sheep that resist *Haemonchus contortus* challenge. Vet. Parasitol. 205, 595–605.

Andronicos, N.M., McNally, J., Kotze, A.C., Hunt, P.W., Ingham, A., 2012. *Trichostrongylus colubriformis* larvae induce necrosis and release of interleukin 33 from intestinal epithelial cells in vitro: implications for gastrointestinal nematode vaccine design. Int. J. Parasitol. 42, 295–304.

Andronicos, N., Hunt, P., Windon, R., 2010. Expression of genes in gastrointestinal and lymphatic tissues during parasite infection in sheep genetically resistant or susceptible to *Trichostrongylus colubriformis* and *Haemonchus contortus*. Int. J. Parasitol. 40, 417–429.

Barger, I.A., 1997. Control by management. Vet. Parasitol. 72, 493–506.

Barger, I.A., 1988. Resistance of young lambs to *Haemonchus contortus* infection, and its loss following anthelmintic treatment. Int. J. Parasitol. 18, 1107–1109.

Barger, I.A., Le Jambre, L.F., 1979. The role of inhibited larvae in the epidemiology of ovine haemonchosis. Aust. Vet. J. 55, 580–583.

Barger, I.A., Le Jambre, L.F., 1988. Regulation of *Haemonchus contortus* populations in sheep: mortality of established worms. Int. J. Parasitol. 18, 269–273.

Barger, I.A., Le Jambre, L.F., Georgi, J.R., Davies, H.I., 1985. Regulation of *Haemonchus contortus* populations in sheep exposed to continuous infection. Int. J. Parasitol. 15, 529–533.

Barrere, V., Falzon, L.C., Shakya, K.P., Menzies, P.I., Peregrine, A.S., Prichard, R.K., 2013. Assessment of benzimidazole resistance in *Haemonchus contortus* in sheep flocks in Ontario, Canada: comparison of detection methods for drug resistance. Vet. Parasitol. 198, 159–165.

Beh, K.J., Hulme, D.J., Callaghan, M.J., Leish, Z., Lenane, I., Windon, R.G., Maddox, J.F., 2002. A genome scan for quantitative trait loci affecting resistance to *Trichostrongylus colubriformis* in sheep. Anim. Genet. 33, 97–106.

Benavides, M.V., Sonstegard, T.S., Kemp, S., Mugambi, J.M., Gibson, J.P., Baker, R.L., Hanotte, O., Marshall, K., van Tassell, C., 2015. Identification of novel loci associated with gastrointestinal parasite resistance in a Red Maasai × Dorper backcross population. PLoS ONE 10, e0122797.

Bendixsen, T., Emery, D.L., Jones, W.O., 1995. The sensitization of mucosal mast cells during infections with *Trichostrongylus colubriformis* or *Haemonchus contortus* in sheep. Int. J. Parasitol. 25, 741–748.

Bendixsen, T., Windon, R.J., Huntley, J.F., MacKellar, A., Davey, R.J., McClure, S.J., Emery, D.L., 2004. Development of a new monoclonal antibody to chimeric ovine IgE and its detection of local and systemic IgE antibody to the intestinal nematode, *Trichostrongylus colubriformis*. Vet. Immunol. Immunopathol. 97, 11–24.

Besier, R.B., Khan, L.W., Sargison, N.D., Van Wyk, J.A., 2016. The pathophysiology, ecology and epidemiology of *Haemonchus contortus* infection in small ruminants. Adv. Parasitol. 93, 95–143.

Besier, R.B., Love, R.A., Lyon, J., van Burgel, A.J., 2010. A targeted selective treatment approach for effective and sustainable sheep worm management: investigations in Western Australia. Anim. Prod. Sci. 50, 1034–1042.

Bishop, S.C., 2012. Possibilities to breed for resistance to nematode parasite infections in small ruminants in tropical production systems. Animal 6 (Special Issue 05), 741–747.

Blitz, N.M., Gibbs, H.C., 1972a. Studies on the arrested development of *Haemonchus contortus* in sheep—I. The induction of arrested development. Int. J. Parasitol. 2, 5–12.

Blitz, N.M., Gibbs, H.C., 1972b. Studies on the arrested development of *Haemonchus contortus* in sheep—II. Termination of arrested development and the spring rise phenomenon. Int. J. Parasitol. 2, 13–22.

Britton, C., Roberts, B., Marks, N.D., 2016. Functional genomics tools for *Haemonchus contortus* and lessons from other helminths. Adv. Parasitol. 93, 599–623.

Casadevall, A., Pirofski, L., 2001. Host-pathogen interactions: the attributes of virulence. J. Inf. Dis. 184, 337–344.

Cerutti, M.C., Citterio, C.V., Bazzocchi, C., Epis, S., D'Amelio, S., Ferrari, N., Lanfranchi, P., 2010. Genetic variability of *Haemonchus contortus* (Nematoda: Trichostrongyloidea) in alpine ruminant host species. J. Helminth. 84, 276–283.

Clunies Ross, I., Gordon, H.Mc.L., 1936. The Internal Parasites and Parasitic Diseases of Sheep. Angus & Robertson Ltd., Sydney, Australia.

Coop, R.L., Huntley, J.F., Smith, W.D., 1995. Effect of dietary protein supplementation on the development of immunity to *Ostertagia circumcincta* in growing lambs. Res. Vet. Sci. 59, 24–29.

Corley, M.M., Jarmon, A.A., 2012. A common Btubulin isotype-1 gene single nucleotide polymorphism as a tool for detection and quantitation of anthelmintic resistant *Haemonchus contortus* in grazing goats. J. Agric. Sci. 4, 1–11.

Cornelius, M.P., Jacobson, C., Dobson, R., Besier, R.B., 2016. Computer modelling of anthelmintic resistance and worm control outcomes for refugia-based nematode control strategies in Merino ewes in Western Australia. Vet. Parasitol. 220, 59–66.

Crofton, H.D., Whitlock, J.H., 1965a. Ecology and biological plasticity of sheep nematodes. IV. The biological significance of temperature x time hatching curves for eggs of sheep nematodes. Cornell Vet. 55, 263–274.

Crofton, H.D., Whitlock, J.H., 1965b. Ecology and biological plasticity of sheep nematodes. V. The relationship between egg volume and hatching time. Cornell Vet. 55, 274–279.

Dash, K.M., 1971. Annual Report. Division of Animal Health, CSIRO, Melbourne, Australia.

Diez-Tacson, C., Keane, O.M., Wilson, T., Zadissa, A., Hyndman, D.L., Baird, D.B., McEwan, J.C., Crawford, A.M., 2005. Microarray analysis of selection lines from outbred populations to identify genes involved with nematode parasite resistance in sheep. Physiol. Genomics 21, 59–69.

Dineen, J.K., Donald, A.D., Wagland, B.M., Offner, J., 1965. The dynamics of the host-parasite relationship III. The response of sheep to primary infection with *Haemonchus contortus*. Parasitology 55, 515–525.

Dobson, R.J., Barnes, E.H., 1995. Interaction between *Ostertagia circumcincta* and *Haemonchus contortus* infection in young lambs. Int. J. Parasitol. 25, 495–501.

Dobson, R.J., Barnes, E.H., Tyrrell, K.L., Hosking, B.C., Larsen, J.W.A., Besier, R.B., Love, S., Rolfe, P.F., Bailey, J.N., 2011. A multi-species model to access the effect of refugia on worm control and anthelmintic resistance in sheep grazing systems. Aust. Vet. J. 89, 200–208.

Dobson, R.J., Waller, P.J., Donald, A.D., 1990. Population dynamics of *Trichostrongylus colubriformis* in sheep; the effect of infection rate and no the establishment of infective larvae and parasite fecundity. Int. J. Parasitol. 20, 347–352.

Donaldson, J., van Houtert, M.F.J., Sykes, A.R., 1998. The effect of nutrition on the periparturient parasite status of mature ewes. Anim. Sci. 67, 523–533.

Doyle, E.K., Kahn, L.P., McClure, S.J., 2014. Nutrient partitioning of Merino sheep divergently selected for genetic difference in resistance to *Haemonchus contortus*. Vet. Parasitol. 205, 175–185.

Ehrenreich, I.M., Bloom, J., Torab, I.N., Wang, X., Jia, Y., Kruglyak, L., 2012. Genetic architecture of highly complex chemical resistance traits across four yeast strains. PLoS Genet. 8, e1002570.

Emery, D.L., 1996. Vaccination against worm parasites of livestock. Vet. Parasitol. 64, 31–45.

Emery, D.L., McClure, S.J., Davey, R.J., 2000. Protection of neonatal lambs against *Haemonchus contortus* by trickle infection of neonates. Parasitol. Int. 49, 165–170.

Emery, D.L., Wagland, B.M., McClure, S.J., 1993. Rejection of heterologous nematodes by sheep immunized with larval or adult *Trichostrongylus colubriformis*. Int. J. Parasitol. 23, 841–846.

Eysker, M., 1981. Experiments on inhibited development of *Haemonchus contortus* and *Ostertagia circumcincta* in sheep in The Netherlands. Res. Vet. Sci. 30, 62–65.

Gaba, S., Gruner, L., Cabaret, J., 2006. The establishment rate of a sheep nematode: revisiting classics using a meta-analysis of 87 experiments. Vet. Parasitol. 140, 302–311.

Gasser, R.B., Schwarz, E.M., Korhonen, P.K., Young, N.D., 2016. Understanding *Haemonchus contortus* better through genomics and transcriptomics. Adv. Parasitol. 93, 519–567.

Ghisi, M., Kaminsky, R., Maser, P., 2007. Phenotyping and genotyping of *Haemonchus contortus* isolates reveals a new putative candidate mutation for benzimidazole resistance in nematodes. Vet. Parasitol. 144, 313–320.

Gibbs, H.C., 1965. Observations on an outbreak of clinical parasitism in ewes during the winter months. Can. Vet. J. 5, 8–11.

Gilbert, S.F., Bosch, T.C.G., Ledon-Rettig, C., 2015. Eco-evo-devo: developmental symbiosis and developmental plasticity as evolutionary agents. Nat. Rev. Genet. 16, 611–622.

Gilleard, J.S., Redman, E., 2016. Genetic diversity and population structure of *Haemonchus contortus*. Adv. Parasitol. 93, 31–68.
Godoy, P., Che, H., Beech, R.N., Prichard, R.K., 2015. Characterization of *Haemonchus contortus* P-glycoprotein-16 and its interaction with the macrocyclic lactone anthelmintics. Mol. Biochem. Parasitol. 204, 11–15.
Hart, E.H., Brophy, P.M., Prescott, M., Bartley, D.J., Wolf, B.T., Hamilton, J.V., 2015. A new enabling proteomics methodology to investigate membrane associated proteins from parasitic nematodes: case study using ivermectin resistant and ivermectin susceptible isolates of *Caenorhabditis elegans* and *Haemonchus contortus*. Vet. Parasitol. 207, 266–275.
Harrison, G.B.L., Pulford, H.D., Doolin, E.E., Pernthaner, A., Shoemaker, C.B., Hein, W. R., 2008. Antibodies to surface epitopes of the carbohydrate larval antigen CarLA are associated with passive protection in strongylid nematode challenge infections. Parasite Immunol. 30, 577–584.
Hein, W.R., Pernthaner, A., Piedrafita, D., Meeusen, E.N., 2010. Immune mechanisms of resistance to gastrointestinal nematode infections in sheep. Parasite Immunol. 32, 541–548.
Hijmans, R.J., Cameron, S.E., Parra, J.L., Jones, P.G., Jarvis, A., 2005. Very high resolution interpolated climate surfaces for global land areas. Int. J. Climatol. 25, 1965–1978.
Hoberg, E.P., Zarlenga, D.S., 2016. Evolution and biogeography of *Haemonchus contortus*: linking faunal dynamics in space and time. Adv. Parasitol. 93, 1–30.
Hendrikx, W.M.L., Bpersema, J.H., Eysker, M., 1988. The influence of cryopreservation on a benzimidazole-resistant isolate of *Haemonchus contortus* conditioned for inhibition. Parasitol. Res. 74, 569–573.
Hunt, P.W., Kotze, A.C., Knox, M.R., Anderson, L.J., McNally, J., Le Jambre, L.F., 2010. The use of DNA markers to map anthelmintic drug resistance loci in an intraspecific cross of *Haemonchus contortus*. Parasitology 137, 705–717.
Hunt, P.W., Knox, M.R., Le Jambre, L.F., McNally, J., Anderson, L.J., 2008. Genetic and phenotypic differences between isolates of *Haemonchus contortus* in Australia. Int. J. Parasitol. 38, 885–900.
Hussain, T., Periasamy, K., Asif, N., Masroor Ellahi, B., Pichler, R., Diallo, A., 2014. Sympatric species distribution, genetic diversity and population structure of *Haemonchus* isolates from domestic ruminants in Pakistan. Vet. Parasitol. 206, 188–199.
Ingham, A., Reverter, A., Windon, R., Hunt, P., Menzies, M., 2008. Gastrointestinal nematode challenge induces some conserved gene expression changes in the gut mucosa of genetically resistant sheep. Int. J. Parasitol. 38, 431–442.
Ingham, A., Menzies, M., Hunt, P., Reverter, A., Windon, R., Andronicos, N., 2011. Divergent ghrelin expression patterns in sheep genetically resistant or susceptible to gastrointestinal nematodes. Vet. Parasitol. 181, 194–202.
Jacobs, H.J., Wiltshire, C., Ashman, K., Meeusen, E.N., 1999. Vaccination against the gastrointestinal nematode, *Haemonchus contortus*, using a purified larval surface antigen. Vaccine 17, 362–368.
Jones, W.O., Huntley, J.F., Emery, D.L., 1992. Isolation and degranulation of mucosal mast cells from the small intestine of parasitized sheep. Int. J. Parasitol. 22, 519–521.
Jones, W.O., Emery, D.L., McClure, S.J., Wagland, B.M., 1994. Changes in inflammatory mediators and larval inhibitory activity in intestinal contents and mucus during primary and challenge infections of sheep with *Trichostrongylus colubriformis*. Int. J. Parasitol. 24, 519–525.
Kaplan, R.M., 2004. Drug resistance in nematodes of veterinary importance: a status report. Trends Parasitol. 20, 477–481.
Kemper, K.E., Emery, D.L., Bishop, S.C., Oddy, H., Hayes, B.J., Dominik, S., Henshall, J. M., Goddard, M.E., 2011. The distribution of SNP marker effects for faecal worm egg count in sheep, and the feasibility of using these markers to predict genetic merit for resistance to worm infections. Genetics Res. 93, 203–219.
Kenyon, F., Greer, A.W., Coles, G.C., Cringoli, G., Papadopoulos, E., Cabaret, J., Berrag, B., Varady, M., Van Wyk, J.A., Thomas, E., Vercruysse, J., Jackson, F., 2009. The role of targeted selective treatments in the development of refugia-based strategies to the control of gastrointestinal nematodes of small ruminants. Vet. Parasitol. 64, 3–11.
Kotze, A.C., Prichard, R.K., 2014. Anthelmintic Resistance in *Haemonchus contortus*: History, Mechanisms and Diagnosis. Adv. Parasitol. 93, 397–428.
Kotze, A.C., Hunt, P.W., Skuce, P., von Samson-Himmelstjerna, G., Martin, R.J., Sager, H., Krücken, J., Hodgkinson, J., Lespine, A., Jex, A.R., Gilleard, J.S., Beech, R.N., Wolstenholme, A.J., Demeler, J., Robertson, A.P., Charvet, C.L., Neveu, C., Kaminsky, R., Rufener, L., Alberich, M., Menez, C., Prichard, R.K., 2014. Recent advances in candidate-gene and whole-genome approaches to the discovery of anthelmintic resistance markers and the description of drug/receptor interactions. Int. J. Parasitol. DDR 4, 164–184.
Kotze, A.C., Cowling, K., Bagnal, L.N.H., Hines, B.M., Ruffell, A.P., Hunt, P.W., Coleman, G.T., 2012. Relative level of thiabendazole resistance associated with the E198A and F200Y SNPs in larvae of a multi-drug resistant isolate of *Haemonchus contortus*. Int. J. Parasitol. DDR 2, 92–97.
Kwa, M.S.G., Kooyman, F.N.J., Boersema, J.H., Roos, M.H., 1993. Effect of selection for benzimidazole resistance in *Haemonchus contortus* on beta-tubulin isotype 1 and isotype 2 genes. Biochem. Biophys. Res. Comm. 191, 413–419.
Laing, R., Martinelli, A., Tracey, A., Holroyd, N., Gilleard, J.S., Cotton, J.A., 2016. *Haemonchus contortus*: genome structure, organisation and comparative genomics. Adv. Parasitol. 93, 569–597.
Laing, R., Bartley, D.J., Morrison, A.A., Rezansoff, A., Martinelli, A., Laing, S.T., Gilleard, J.S., 2015. The cytochrome p450 family in the parasitic nematode *Haemonchus contortus*. Int. J. Parasitol. 45, 243–251.
Laing, R., Kikuchi, T., Martinelli, A., Tsai, I.J., Beech, R.N., Redman, E., Holroyd, N., Bartley, D.J., Beasley, H., Britton, C., Curran, D., Devaney, E., Gilabert, A., Hunt, M., Jackson, F., Johnston, S., Kryukov, I., Keyu, Li., Morrison, A.A., Reid, A.J., Sargison, N., Saunders, G., Wasmuth, J.D., Wolstenholme, A., Berriman, M., Gilleard, J.S., 2013. The genome and transcriptome of *Haemonchus contortus*, a key model parasite for drug and vaccine discovery. Genome Biol. 14, R88.
Lane, J., Jubb, T., Shephard, R., Webb-Ware, J., Fordyce, G., 2015. MLA Final Report: Priority List of Endemic Diseases for the Red Meat Industries. Meat and Livestock Australia, Sydney, Australia. http://www.wormboss.com.au/files/pages/worms/roundworms/the-cost-of-roundworms/B.AHE.0010_Final_Report.pdf.
Law, W.J., Wuescher, L.M., Ortega, A., Hapiak, V.M., Komuniecki, P.R., Komuniecki, R., 2015. Heterologous expression in remodeled *C. elegans*: a platform for monoaminergic agonist identification and anthelmintic screening. PLoS Pathog. 11, e1004794.
Lecova, L., Ruzickova, M., Laing, R., Vogel, H., Szotakova, B., Prchal, L., Lamka, J., Vokral, I., Skalova, L., Matouskova, P., 2015. Reliable reference gene selection for quantitative real time PCR in *Haemonchus contortus*. Mol. Biochem. Parasitol. 201, 123–127.
Le Jambre, L.F., 1972. Optimum temperature for egg development of phenotypes in *Haemonchus contortus cayugensis* as determined by Arrhenius diagrams and Sacher's entropy function. Int. J. Parasitol. 3, 299–310.
Le Jambre, L.F., 1981. Hybridization of Australian *Haemonchus placei* (Place, 1893), *Haemonchus contortus cayugensis* (Das & Whitlock, 1960) and *Haemonchus contortus* (Rudolphi, 1803) from Louisiana. Int. J. Parasitol. 11, 323–330.
Le Jambre, L.F., Dobson, R.J., Lenane, I.J., Barnes, E.H., 1999. Selection for anthelmintic resistance by macrocyclic lactones in *Haemonchus contortus*. Int. J. Parasitol. 29, 1101–1111.
Le Jambre, L.F., Ratcliffe, L.H., Whitlock, J.H., Crofton, H.D., 1970. Polymorphism and egg-size in the sheep nematode, *Haemonchus contortus*. Evolution (N.Y.) 24, 625–631.
Le Jambre, L.F., Royal, W.M., 1977. Genetics of vulvar morph types in *Haemonchus contortus*: *Haemonchus contortus* from the Northern tablelands of New South Wales. Int. J. Parasitol. 7, 481–487.
Le Jambre, L.F., Windon, R.G., Smith, W.D., 2008. Vaccination against *Haemonchus contortus*: performance of native parasite gut membrane glycoproteins in Merino lambs grazing contaminated pasture. Vet. Parasitol. 153, 302–312.
Macarthur, F.A., Kahn, L., Windon, R.G., 2014. The influence of dietary manipulations and gastrointestinal nematodes on twin-bearing merino ewes and determinants of lamb survival. Livestock Sci. 157, 342–352.
McClure, S.J., Emery, D.L., Wagland, B.M., Jones, W.O., 1992. A serial study of rejection of *Trichostrongylus colubriformis* by immune sheep. Int. J. Parasitol. 22, 227–234.
McClure, S.J., McClure, T.J., Emery, D.L., 1999. Effects of molybdenum intake on primary infection and subsequent challenge by the nematode parasite *Trichostrongylus colubriformis* in weaned Merino lambs. Res. Vet Sci. 67, 17–22.
McKenna, P.B., 1973. The significance of inhibition in the parasitic development of abomasal nematodes in New Zealand sheep. N. Z. Vet. J. 21, 98–102.
McNeilly, T.N., McClure, S.J., Huntley, J.F., 2008. Mucosal immunity in sheep and implications for mucosal vaccine development. Small Ruminant Res. 76, 83–91.
Medica, D.L., Sukhdeo, M.V.K., 2001. Estimating transmission potential in gastrointestinal nematodes (order: Strongylida). J. Parasitol. 87, 442–445.
McRae, K.M., McEwan, J.C., Dodds, K.G., Gemmell, N.J., 2014. Signatures of selection in sheep bred for resistance or susceptibility to gastrointestinal nematodes. BMC Genet. 15, 637–645.
Meeusen, E.N., Balic, A., Bowles, V., 2005. Cells, cytokines and other molecules associated with rejection of gastrointestinal nematode parasites. Vet. Immunol. Immunopathol. 108, 121–125.
Michel, J.F., 1970. The regulation of populations of *Ostertagia ostertagi* in calves. Parasitology 61, 435–447.
Miller, H.R.P., 1984. The protective mucosal response against gastrointestinal nematodes in ruminants and laboratory animals. Vet. Immun. Immunopath. 6, 167–259.
Mohandas, N., Young, N.D., Jabbar, A., Korhonen, P.K., Koehler, A.V., Hall, R.S., Hu, M., Hofmann, A., Gasser, R.B., 2016. The complement of family M1 aminopeptidases of *Haemonchus contortus* - biotechnological implications. Biotech. Adv. 34, 65–76.
Morley, F.H.W., Donald, A.D., 1980. Farm management and systems of helminth control. Vet. Parasitol. 6, 105–134.
Morris, C.A., Bisset, S.A., Vlassoff, A., West, C.J., Wheeler, M., 1998. Faecal nematode egg counts in lactating ewes from Romney flocks selectively bred for divergence in lamb faecal egg count. Anim. Sci. 67, 283–288.
Mugambi, J.M., Bain, R.K., Wanyangu, S.K., Ihiga, M.A., Duncan, J.L., Murray, M., Stear, M.J., 1997. Resistance of four sheep breeds to natural and subsequent artificial *Haemonchus contortus* infection. Vet. Parasitol. 69, 265–273.
Munn, E.A., Greenwood, C.A., Coadwell, W.J., 1987. Vaccination of young lambs by means of a protein fraction extracted from adult Haemonchus contortus. Parasitology 94, 385–397.
Mutwiri, G., Bateman, C., Baca-Estrada, M.E., Snider, M., Griebel, P., 2000. Induction of immune responses in newborn lambs following enteric immunization with a human adenovirus vaccine vector. Vaccine 19, 1284–1293.
Nisbet, A.J., Meeusen, E.N., Gonzalez, J.F., Piedrafita, D.M., 2016. Immunity to *Haemonchus contortus* and vaccine development. Adv. Parasitol. 93, 353–396.
Otsen, M., Plas, M.E., Lenstra, J.A., Roos, M.H., Hoekstra, R., 2000. Microsatellite diversity of isolates of the parasitic nematode *Haemonchus contortus*. Mol. Biochem. Parasitol. 110, 69–77.
Otsen, M., Hoekstra, R., Plas, M.E., Buntjer, J.B., Lenstra, J.A., Roos, M.H., 2001. Amplified fragment length polymorphism analysis of genetic diversity of

Haemonchus contortus during selection for drug resistance. Int. J. Parasitol. 31, 1138–1143.
Parkinson, J., Mitreva, M., Whitton, C., Thomson, M., Daub, J., Martin, J., Schmid, R., Hall, N., Barrell, B., Waterston, R.H., McCarte, R.J.P., Blaxter, M.L., 2004. A transcriptomic analysis of the phylum Nematoda. Nat. Genet. 36, 1259–1267.
Pernthaner, A., Shaw, R.J., McNeill, M.M., Morrison, L., Hein, W.R., 2005a. Total and nematode-specific IgE responses in intestinal lymph of genetically resistant and susceptible sheep during infection with *Trichostrongylus colubriformis*. Vet. Immunol. Immunopathol. 104, 69–80.
Pernthaner, A., Cole, S.A., Morrison, L., Hein, W.R., 2005b. Increased expression of interleukin-5 (IL-5), IL-13, and tumor necrosis factor alpha genes in intestinal lymph cells of sheep selected for enhanced resistance to nematodes during infection with *Trichostrongylus colubriformis*. Inf. Immun. 73, 2175–2183.
Poeschel, G.P., Todd, A.C., 1972a. Selection for variations in pathogenicity of *Haemonchus contortus* isolates. Am. J. Vet. Res. 33, 1575–1582.
Poeschel, G.P., Todd, A.C., 1972b. Disease-producing capacity of *Haemonchus contortus* isolates in sheep. Am. J. Vet. Res. 33, 2207–2213.
Porco, T.C., Lloyd-Smith, J.O., Gross, K.L., Galvani, A.P., 2005. The effect of treatment on pathogen virulence. J. Theoret. Biol. 233, 91–102.
Procter, B.G., Gibbs, H.C., 1968. Studies on the spring rise phenomenon in ovine helminthiasis I. spring rise in stabled sheep. Can. J. Comp. Med. Vet. Sci. 32, 359–365.
Redman, E., Packard, E., Grillo, V., Smith, J., Jackson, F., Gilleard, J.S., 2008. Microsatellite analysis reveals marked genetic differentiation between *Haemonchus contortus* laboratory isolates and provides a rapid system of genetic fingerprinting. Int. J. Parasitol. 38, 111–122.
Redman, E., Sargison, N., Whitelaw, F., Jackson, F., Morrison, A., Bartle, Y.D.J., Gilleard, J.S., 2012. Introgression of ivermectin resistance genes into a susceptible Haemonchus contortus strain by multiple backcrossing. PLoS Pathog. 2, e1002534.
Reinecke, R.K., Snyman, H.M., Seaman, H., 1979. Studies on *Haemonchus contortus* II. The effect of abomasal nematodes on subsequent challenge with *H. contortus*. Onderstepoort J. Vet. Res. 46, 199–205.
Reinecke, R.K., Bruckner, C., De Villiers, I.L., 1981. Studies on *Haemonchus contortus*. IV. The effect of *Trichostrongylus axei* and *Ostertagia circumcincta* on challenge with *H. contortus*. Onderstepoort J. Vet. Res. 48, 229–234.
Roos, M.H., Otsen, M., Hoekstra, R., Veenstra, J.G., Lenstra, J.A., 2004. Genetic analysis of inbreeding of two strains of the parasitic nematode *Haemonchus contortus*. Int. J. Parasitol. 34, 109–115.
Rose, H., Hoar, B., Kutz, S.J., Morgan, E.R., 2014. Exploiting parallels between livestock and wildlife: predicting the impact of climate change on gastrointestinal nematodes in ruminants. Int. J. Parasitol. PAW. 3, 209–219.
Rowe, A., McMaster, K., Emery, D.L., Sangster, N., 2008. *Haemonchus contortus* infection in sheep: parasite fecundity correlates with worm size and host lymphocyte counts. Vet. Parasitol. 153, 285–293.
Rowe, A., Gondro, C., Emery, D.L., Sangster, N., 2009. Sequential microarray to identify timing of molecular responses to *Haemonchus contortus* infection in sheep. Int. J. Parasitol. 161, 76–87.
Sarai, R.S., Kopp, S.R., Knox, M.R., Coleman, G.T., Kotze, A.C., 2015. *In vitro* levamisole selection pressure on larval stages of *Haemonchus contortus* over nine generations gives rise to drug resistance and target site gene expression changes specific to the early larval stages only. Vet. Parasitol. 211, 45–53.
Sargison, N.D., Jackson, F., Bartley, D.J., Wilson, D.J., Stenhouse, L.J., Penny, C.D., 2007. Observations on the emergence of multiple anthelmintic resistance in sheep flocks in the south-east of Scotland. Vet. Parasitol. 145, 65–76.
Schwarz, E.M., Korhonen, P.K., Campbell, B.E., Young, N.D., Jex, A.R., Jabbar, A., Hall, R.S., Mondal, A., Howe, A.C., Pell, J., Hofmann, A., Boag, P.R., XingQuan, Zh.u., Gregory, T.R., Loukas, A., Williams, B.A., Antoshechkin, I., Brown, C.T., Sternberg, P.W., Gasser, R.B., 2013. The genome and developmental transcriptome of the strongylid nematode *Haemonchus contortus*. Genome Biol. 14, R89.
Senel, S., McClure, S.J., 2004. Potential applications of chitosan in veterinary medicine. Adv. Drug Deliv. Rev. 56, 1467–1480.
Shamaila, I., Mazhar, Q., Donskow-Lysoniewska, K., Zia-ul-Haq, M., Stear, M.J., 2014. Genetic variability in beta-tubulin-1 in benzimidazole resistant *Haemonchus contortus* from sheep in north-east Punjab, Pakistan. Pak. J. Zool. 46, 431–435.
Silvestre, A., Sauve, C., Cortet, J., Cabaret, J., 2009. Contrasting genetic structures of two parasitic nematodes, determined on the basis of neutral microsatellite markers and selected anthelmintic resistance markers. Mol. Ecol. 18, 5086–5100.
Smeal, M.G., Fraser, G.C., Robinson, G.G., 1980. Seasonal changes in the structure of nematode populations of cattle in New South Wales in relation to inhibited larval development. Aust. Vet. J. 80, 80–86.
Smith, W.D., Angus, K.W., 1980. *Haemonchus contortus*: attempts to immunise lambs with irradiated larvae. Res. Vet. Sci. 29, 45–50.
Smith, W.D., Jackson, F., Jackson, E., Williams, J., 1983. Local immunity and *Ostertagia circumcincta*: changes in the gastric lymph of immune sheep after a challenge infection. J. Comp. Path. 93, 479–488.
Smith, W.D., Smith, S.K., Newlands, G.F., Skuce, P.J., 2000. Relative protective properties of three membrane glycoprotein fractions from *Haemonchus contortus*. Parasite Immunol. 22, 63–71.
Southcott, W.H., Major, G.W., Barger, I.A., 1976. Seasonal pasture contamination and availability of nematodes for grazing sheep. Aust. J. Agric. Res. 27, 277–286.
Stear, M.J., Bishop, S.C., Doligalska, M., Duncan, J.L., Holmes, P.H., Irvine, J., McCririe, L., McKellar, Q.A., Sinski, E., Murray, M., 1995. Regulation of egg production, worm burden, worm length and worm fecundity by host responses in sheep infected with *Ostertagia circumcincta*. Parasite Immunol. 17, 643–652.
Stewart, D.F., 1955. 'Self-cure' in nematode infections of sheep. Nature 176, 1273–1274.
Strain, S.A.J., Stear, M.J., 2001. The influence of protein supplementation on the immune response to *Haemonchus contortus*. Parasite Immunol. 23, 527–531.
Stroehlein, A.J., Young, N.D., Korhonen, P.K., Jabbar, A., Hofmann, A., Sternberg, P.W., Gasser, R.B., 2015. The *Haemonchus contortus* kinome – a resource for fundamental molecular investigations and drug discovery. Parasites Vectors 8, 623–630.
Troell, K., Engstrom, A., Morrison, D.A., Mattsson, J.G., Hoglund, J., 2006. Global patterns reveal strong population structure in *Haemonchus contortus*, a nematode parasite of domesticated ruminants. Int. J. Parasitol. 36, 1305–1316.
Turner, J.H., Kates, K.C., Wilson, G.I., 1962. The interaction of concurrent infections of the abomasal nematodes, *Haemonchus contortus, Ostertagia circumcincta* and *Trichostrongylus axei* (Trichostrongylidae) in lambs. Proc. Helminth. Soc. Washington 20, 210–216.
Urdaneta-Marquez, L., Bae, S.H., Janukavicius, P., Beech, R., Dent, J., Prichard, R., 2014. A *dyf*-7 haplotype causes sensory neuron defects and is associated with macrocyclic lactone resistance worldwide in the nematode parasite *Haemonchus contortus*. Int. J. Parasitol. 44, 1063–1071.
Van Den Brom, R., Moll, L., Kappert, C., Vellema, P., 2015. *Haemonchus contortus* resistance to monepantel in sheep. Vet. Parasitol. 209, 278–280.
Van Houtert, M.F.J., Barger, I.A., Steel, R.G., Windon, R.G., Emery, D.L., 1995. Effects of dietary protein intake on responses of young sheep to infection with *Trichostrongylus colubriformis*. Vet. Parasitol. 56, 163–180.
Van Wyk, J.A., Van Schalkwyk, P.C., 1990. A novel approach to the control of anthelmintic-resistant *Haemonchus contortus* in sheep. Vet. Parasitol. 35, 61–69.
Waller, P.J., Thomas, R.J., 1975. Field studies on inhibition of *Haemonchus contortus* in sheep. Parasitology 71, 285–291.
Waller, P.J., Rudby-Martin, L., Ljungström, B.L., Rydzika, A., 2004. The epidemiology of abomasal nematodes of sheep in Sweden, with particular reference to over-winter survival strategies. Vet. Parasitol. 122, 207–220.
Walsh, T.K., Donnan, A.A., Jackson, F., Skuce, P., Wolstenholme, A.J., 2007. Detection and measurement of benzimidazole resistance alleles in *Haemonchus contortus* using real-time PCR with locked nucleic acid Taqman probes. Vet. Parasitol. 144, 304–312.
Willadsen, P., 2004. Anti-tick vaccines. Parasitology 129, S367–S387.
Williamson, S.M., Storey, B., Howell, S., Harper, K.M., Kaplan, R.M., 2011. Candidate anthelmintic resistance-associated gene expression and sequence polymorphisms in a triple-resistant field isolate of *Haemonchus contortus*. Mol. Biochem. Parasitol. 180, 99–105.
Wilson, G.I., Samson, K.S., 1970. Clinical haemonchosis from maturation of inhibited larvae. JAVMA 156, 1869–1870.
Windon, R.G., 1996. Genetic control of resistance to helminths in sheep. Vet. Immunol. Immunopathol. 54, 245–254.
Woolaston, R.R., Piper, L.R., 1996. Selection of Merino sheep for resistance to *Haemonchus contortus*: genetic variation. Anim. Sci. 62, 451–460.
Woolaston, R.R., Windon, R.G., 2001. Selection of sheep for response to *Trichostrongylus colubriformis* larvae: genetic parameters. Anim. Sci. 73, 41–48.
Yin, F.Y., Gasser, R.B., Li, F.C., Bao, M., Huang, W.Y., Zou, F.C., Zhao, G.H., Wang, C.R., Yang, X., Zhou, Y.Q., Zhao, J.L., Fang, R., Hu, M., 2013. Genetic variability within and among *Haemonchus contortus* isolates from goats and sheep in China. Parasites Vectors 6, 279–286.